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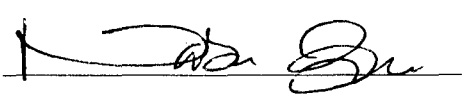

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13. Abstract ***(Maximum 200 Words)*** *(abstract should contain no proprietary or confidential information)*

The entire coding regions and flanking introns of BRCA2 have been screened for germline mutations in 74 high-risk African American breast cancer patients. Eight protein truncating, pathogenic mutations have been encountered in female and male patients. Four (1991delATAA, 1993delAA, 2001delTTAT, 8643delAT) of the pathogenic mutations observed in African Americans have not been previously described and may be unique to this group. Six novel rare variations were observed in patients. Of eleven polymorphisms identified in patients, four may be unique to African Americans. The difference in heterozygosity between patients and African American controls for the intron 10 variation is statistically significant. Neither the *BRCA2* pathogenic mutations, rare variations, nor two of the polymorphisms have been detected in 163 disease-free control subjects. Since many different mutations/variations are observed in African Americans, BRCA2 genetic testing in high-risk African American families must include the entire coding and flanking non-coding regions of the gene. This study supports the importance of early-onset breast cancer (< 40), male breast cancer, multiple cases of breast cancer with at least one before age 50, ovarian cancer, and prostate cancer in a family as indicators for BRCA genetic testing in African Americans.

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INTRODUCTION

Mutations in single genes account for 3-8% of all breast cancer (Easton, 1994; Eeles et al., 1994). Early onset disease is common in hereditary breast cancer families. The incidence of breast cancer among blacks is higher than whites below the age of fifty (Ries et al., 1998). Most inherited breast cancer is due to the highly penetrant breast cancer predisposing genes *BRCA1* and *BRCA2*. Specific breast cancer predisposing mutations in *BRCA1* and *BRCA2* have been associated with different ethnic groups; therefore mutations in African Americans are expected to differ from those reported in other populations. Most early studies of hereditary breast cancer have included few African Americans (Miki et al., 1994; Futreal et al. 1994; Castilla et al., 1994). In order to determine the spectrum of germline *BRCA1* and *BRCA2* mutations in African Americans, the entire coding regions and flanking introns are being examined in 80 breast cancer patients from families at high-risk of hereditary breast cancer. Thus, this study represents one of the largest collections of high-risk African Americans whose *BRCA1* and *BRCA2* coding sequences are being completely scanned for mutations. Mutations/variations detected in high-risk families are also being tested in controls, unselected for disease. This investigation will provide information for breast cancer genetic testing and genetic counseling in African Americans.

BODY

Task 4: To collect more blood samples from high-risk African American breast cancer patients, low-risk patients and controls. High-risk criteria are multiple cases (including first-degree, second-degree, and distant relatives in the same lineage) of breast cancer or multiple cases of breast and ovarian cancer per family; or breast cancer with early age of onset ($\leq$ 40 years); or bilateral breast cancer; or breast and ovarian cancer in the same individual; or male breast cancer. During the award period, we have recruited: 43 affected and unaffected members from 25 high risk families; 6 low-risk breast cancer patients, who do not meet the high-risk criteria; and 90 control subjects, who do not have breast cancer or belong to high risk families. Therefore, the total number of high-risk families is 91; the total number of control subjects is 180. There are 90 low-risk patients.

Task 1: To identify *BRCA1* mutations in additional high-risk African American families. *BRCA1* mutation analysis using single-stranded conformational polymorphism (SSCP) followed by sequencing of variants in high-risk African American families has been extended from 45 families **(Panguluri et al., 1999; Mefford et al., 1999)** to 55 families. Several amino acid and intron 7 polymorphisms were detected; these are probably not disease associated **(Table 1)**. Seventy-seven high-risk families are currently being screen for *BRCA1* mutations using denaturing high performance liquid chromatography (DHPLC). In a blinded comparison DHPLC has recently been reported to be the most reliable method for *BRCA* mutation detection besides DNA sequencing, which is very expensive (Eng et al., 2001). We have worked out the DHPLC conditions for *BRCA1* and have unlimited access to an apparatus on the same floor as the principal investigator's laboratory.

Task 2: To identify *BRCA2* mutations in high-risk African American families. *BRCA2* mutation analysis of the entire coding sequence and flanking introns has been completed in 74 high-risk African American families, using the protein truncation test (PTT), SSCP, and DHPLC followed by sequencing of variants **(Whitfield-Broome et al., 1999; Whitfield-Broome et al., 2000; Kanaan et al., 2000a-e; Olopade et al., 2001; Kanaan, 2001; Kanaan et**

al., 2002 see Appendix). Presentations have been made at numerous scientific meetings; see section on Reportable Outcomes. The methods used will not detect nucleotide substitutions(missense mutations) in the central regions of exons 10 and 11 or large deletions greater than 1 kb.

Table 1 *BRCA1* sequence alterations in African American breast cancer patients.
(*BR*, breast; number in *parentheses* indicates number of breast and ovarian cancer cases per family)

Case number	Cancer type	Age at diagnosis (years)	Exon	Nucleotide[a] /codon	Mutation	Amino acid change	Designation
Polymorphisms (≥1% of chromosomes in this or other studies) and **Noncoding variations**							
BC048	BR(2)	40	11	1256/379	T to G	Ile to Met	I379M
BC051	BR(2)	38	11	1256/379	T to G	Ile to Met	I379M
BC052	BR(2)	33	11	1186/356	A to G	Gln to Arg	Q356R
			11	2576/819	C to A	Ser to Tyr	S819Y
			Intron 7	-	C to T	Non-coding	IVS7+553C/T
BC054	BR(6)	76	11	2576/819	C to A	Ser to Tyr	S819Y

[a]Numbering starting with the first nucleotide in the 5'-untranslated region of *BRCA1* cDNA (GenBank accession no. U14680).
[b]Including newly inserted/deleted amino acids and stop codon

Eight (11%) different *BRCA2* pathogenic germ line mutations were identified in 74 high-risk African American breast cancer or breast-ovarian cancer families but not in 163 disease-free control subjects **(Table 2)**. All of the pathogenic mutations delete 18-84% of the protein including important functional domains such as Rad51 binding site and nuclear localization sequence. Four (1991delATAA, 1993delAA, 2001delTTAT, 8643delAT) of the eight pathogenic mutations observed in African Americans have not been previously described. Therefore, half of the pathogenic mutations observed may be unique to African Americans. The other four pathogenic mutations (1882delT, 2816insA, 4075delGT, 4088delA) detected in African Americans have been previously reported. Two of the pathogenic mutations, 1991delATAA and 2816insA, were detected in male breast cancer patients, consistent with previous studies showing an association of *BRCA2* with male breast cancer. This result is the first reported among African American males. Only one of the eight pathogenic mutations, 2816insA, is recurrent in another African American family. Eighty percent of the pathogenic mutations in female breast cancer patients were identified in women diagnosed with breast cancer under the age of 40 with or without a family history of the disease. All breast or ovarian female probands with *BRCA2* mutations were diagnosed before the age of 45. The median family age of diagnosis of female breast cancer among African American *BRCA2* carriers was 43, not significantly different from that (45.1) in Caucasians (Schubert et al., 1997).

Table 2 ***BRCA2*** **pathogenic mutations in African American breast and ovarian cancer patients**

Family No.	Proband age of diagnosis[a]	Median family age Dx female BR	Exon/ intron	Mutation (nucleotide no.)[b]	Effect (codon)[c]	No. individuals		Other cancers in family
						BR	OV	
BC078	43	51	10	1882delT	Frameshift stop at codon 557	8	0	Stomach, throat
MBC021	45		10	1991delATAA	Frameshift stop at codon 613	4	0	none
BC076	37/45	37	10	1993delAA	Frameshift stop at codon 595	7	1	Stomach, lung Colon, prostate
BC051	38	40	10	2001delTTAT	Frameshift Stop at codon 613	2	0	Lung
MBC029	59		11	2816insA	Frameshift stop at codon 879	1	0	Prostate, pancreatic,brain
OV061	44	57	11	4075delGT	Frameshift stop at codon1285	3	2	none
BC002	37	37	11	4088delA	Frameshift stop at codon 1292	1	0	Prostate colon, vagina
BC001	33	33	19	8643delAT T8642C	Frameshift stop at codon 2811 L2805S	1	0	Stomach, uterine

[a]Individual tested with breast or ovarian cancer (BC, female breast cancer; MBC, male breast cancer; OV, ovarian; BR, female or male breast cancer; Dx, diagnosis).
[b]Numbering starting with the first nucleotide in the 5'-untranslated region of *BRCA2* cDNA (GenBank accession no. U43746),
3' most designation for deletion and addition.
[c]Stop codons including newly inserted/deleted amino acids. Amino acid change designated by codon.

Table 3 *BRCA2* rare variations in African American breast cancer patients[a]

Family No.	Proband age at diagnosis[b]	Median family age Dx female BR	Exon/ intron	Variation (Nucleotide no.) [c]	Effect[d]	No. cancers BR	No. cancers OV	Other Cancers
BC049	67	67	Ex 2	A214C	Noncoding 5'UTR	3	0	Colon
BC038	64/68	57	Ex 3	T459G	Silent, T77T	2	0	Leukemia
	58/59	58	In 7	IVS7+611delCTTAA	Noncoding	1Bi	0	none
BC023	62	62	In 26	IVS26+24A/G IVS26+133T/G	noncoding	3	0	Skin Uterine Bone Stomach

[a]Rare variants (detected by SSCP) are those occurring with a frequency of <1 out of 100 chromosomes.

[b]Individual tested with breast cancer (BR, breast; Dx, diagnosis).

[c]Numbering starting with the first nucleotide in the 5'-untranslated region (UTR) of *BRCA2* cDNA (GenBank accession no. U43746). IVS, intervening sequence; +, number of nucleotides into the intron; intron 7 is 3' to exon 7.

[d]Amino acid change designated by codon.

Table 4 ***BRCA2*** **polymorphisms[a] in African Americans and Global populations**

Exon/ Intron	[b]Nucleotide change	Effect	AA patients heterozygosity[c]	AA control heterozygosity	African controls heterozygosity[d]	Global population heterozygosity[e]
Ex 2	G203A	noncoding 5'UTR	0.150 (18/120)	0.11 (36/326)	0	0.27
Ex 2	C218T	noncoding 5'UTR	0.017 (2/120)	<0.003 (0/326)	0	Latin American, Carribbean; No frequency (BIC)
In10	IVS10+22del T	noncoding	0.057 (7/122)	<0.003) (0/326)	0	<0.003
In 11Q[f]	IVS11+80del TTAA	noncoding	0.154 (21/136)	0.147 (20/136)	0.19	0.34
In 12	IVS12+3delT	noncoding (not splicing)	0.048 (6/126)	0.046 (15/326)	0	<0.003
Ex14A[f]	A7470G	Silent, Serine S2414S	0.150 (21/140)	0.215 (37/172)	0.19	0.25

Table 4 (cont) *BRCA2* polymorphisms[a] in African Americans and Global populations

Exon/ Intron	Nucleotide change[b]	Effect	AA patient heterozygosity[c]	AA control heterozygosity	African controls heterozygosity[d]	Global population heterozygosity[e]
Ex14B[f]	C7625T	Ala2466 Val	0.107 (12/112)	0.097 (17/174)	0.018	Latin American, Carribean, African American, Caucasian, no frequency (BIC)
In 14B[f]	IVS14+53C>T	noncoding	0.045 (5/112)	0.096 (17/176)	0.190	0.152
In 15[f]	IVS15+5C>T IVS15+7C>T	Noncoding noncoding	0.21 (31/144)	0.198 (27/136)	0	0
In 21[g]	IVS21+495T>C	noncoding	0.241 (28/116)	0.241 (42/174)	0.381	0.218

[a]Polymorphisms, variants occurring with a frequency of ≥ 1 out of 100 chromosomes

[b]Numbering starting with the first nucleotide in the 5'-untranslated region of *BRCA2* cDNA. IVS, intervening sequence; +, number of nucleotides into the intron; intron 12, follows exon 12.

[c]The heterozygosity was determined by dividing the diallelic variant chromosomes by the total chromosomes (numbers in parentheses).

[d]Heterozygosity for 21 African control subjects from 11 different populations throughout Africa (Wagner et al., 1999a).

[e]Hetrozygosity for 95 control subjects from Africa, Asia, Europe, Pacific islands, Native American and 71 Australian breast/ovarian patients (Wagner et al., 1999a).

[f]Detected with DHPLC (both In 15 variations are homozygous).

[g]The allelic frequency is 0.448 (52/116) for patients and 0.436 (76/174) for African American control subjects.

Six *BRCA2* rare variations, which occur at a frequency of less than 1 in 100 chromosomes, were identified in five breast cancer patients but not in 163 disease-free control subjects **(Table 3)**. None of these rare variations, which occur in the non-coding regions or result in a silent amino acid change, have been previously reported. Eleven *BRCA2* polymorphisms, which occur at a frequency of greater than 1 in 100 chromosomes, were identified in high-risk African American breast cancer patients **(Table 4)**. Four of these polymorphisms (IVS10+22delT, IVS12+3delT, IVS15+5C>T, IVS15+7C>T) have not been reported and may be unique to African Americans. Two polymorphisms were not observed in 163 disease-free controls. The heterozygosity frequency between patients and controls was not statistically significant (two sample T-test) for 10 of the 11 *BRCA2* polymorphisms, indicating that patients and controls are well matched. The difference in heterozygosity between patients and African American controls for IVS10+22delT is statistically significant, 5.7% (95%, confidence interval [CI] 1.5%, 9.8%), using a two sample T-test. Therefore, the intron 10 variation may be disease related by controlling splicing or regulation; further studies are needed. Many of these variants have been reported previously to occur in African controls and globally in control populations **(Table 4)** (Wagner et al., 1999; Breast Cancer Information Core [BIC]).

Sequencing of DNA from 22 different African American and Jewish controls and patients revealed a homozygous 16 base pair deletion, GGT GTT CTC ATA AAC A, at the intron 15/ exon 16 boundary, compared to the Genbank database. This deletion creates an in-frame splice site and appears to be a more common variant than the Genbank sequence.

Task 4: To screen control subjects and low-risk breast cancer patients for *BRCA* mutations detected in high-risk African American patients. All mutations and variations have been tested in 163 disease-free control samples as discussed under Task 2. There was not time to screen low-risk patients.

Task 3: To functionally test *BRCA1* and *BRCA2* missense or intron mutations/variations that are not detected in controls. This has not been undertaken because of (1) time limitations and (2) no mutations in conserved sites that clearly segregate with disease have been identified.

Task 1 and 4: To write manuscripts and make presentations at scientific meetings. See reportable outcomes.

Discussion.

More deleterious *BRCA2* mutations were identified in this study than in any previous study of blacks. Sixty-three percent (5/8) of the pathogenic mutations occur in families with multiple cases of breast or ovarian cancers among first degree relatives. Although three *BRCA2* carriers (BC001, BC002, MBC029) reported no family history of breast/ovarian cancer and one carrier (BC051) had only two cases of breast/ovarian cancer in her family, mutations were detected among this group in a male breast cancer patient (MBC029) and in females with early onset breast cancer, diagnosed before age 40. It is possible that a reluctance of family members to discuss their diseases contributed to three *BRCA2* carriers reporting no breast/ovarian family history.

BRCA2 carriers (BC001, BC002, MBC029; selected for early age of onset or male breast cancer) not reporting a family history of breast or ovarian cancer did have a family history of other cancers (Table 2). Thus, all *BRCA2* carrier families exhibited multiple cases of breast/ovarian cancers or other cancers. Other cancers observed in these African American

BRCA2 families and reported previously in Caucasian *BRCA2* families are ovarian, prostate, colon, pancreas, stomach, and throat (Berman et al., 1996; BIC)

Among our African American high-risk families, prostate cancer was present in 38% (3/8) of *BRCA2* mutation-positive families, compared with 50% (1/2) of *BRCA1* families (Panguluri et al., 1999) and 17% (11/64) of families without *BRCA1/2* mutations. Others have noted a statistically significant increased risk of prostate cancer among white *BRCA1/2* carrier families (Struewing et al., 1997; Johannsson et al., 1999; BIC). Therefore, multiple cases of breast, ovarian and prostate cancer in a family may be a strong indicator for genetic testing.

In this study, the percent of *BRCA2* mutations in all families is 11% (8/74), and in all female breast cancer or ovarian cancer families is 8% (6/72). Among families with four or more cases of male or female breast cancer or ovarian cancer, the percent of *BRCA2* mutations in African Americans is 17% (4/23), compared to 13% (6/48) in Caucasian families (Schubert et al., 1997). The frequency of *BRCA2* mutations among African American women with breast cancer before age 50 or ovarian cancer at any age and at least one first degree or second degree relative with either diagnosis was the same (11.4%; 4/35) as in primarily Caucasian women (11.5%; 23/200) (Frank et al., 1998). Although the numbers for African Americans are low, it appears that the frequency of *BRCA2* mutations is about the same in blacks as in whites.

Our original hypothesis was that by studying a large number of high-risk African Americans with a family history of breast/ovarian cancer or early-onset breast cancer, we would detect the *BRCA1* and *BRCA2* mutations that are most common in the African American population. We expected that mutations in African Americans would differ from those reported in other populations. Our data support this hypothesis. Fifty percent of our *BRCA2* mutations are unique to the African American population; about 57% of African American *BRCA1/2* mutations reported by all investigators are unique to this group (Olopade et al., 2001)

One purpose of this research was to provide information for genetic testing and counseling. Considering our work and that of others, many different pathogenic mutations and many variants of unknown significance are observed in African Americans. The broad spectrum of mutations/variations in African Americans is explainable by the higher level of genetic diveristy among people of African ancestry (Jorde et al. 1998; Wagner et al., 1999), different geographic origins of ancestors, and genetic admixture. Therefore, genetic testing in African Americans must include the entire coding and flanking non-coding regions of the *BRCA2* gene. This study supports the importance of early-onset breast cancer ($\leq$ 40), multiple cases of breast cancer with at least one before age 50, ovarian cancer, male breast cancer, and prostate cancer in a family as indicators for genetic testing in African Americans.

KEY RESEARCH ACCOMPLISHMENTS

- Additional high-risk families and disease-free control subjects have been recruited; there are now 91 high-risk families and 180 controls
- Two *BRCA1* pathogenic mutations have been detected; one of which is a founder mutation in five families of west African ancestry.
- Eight different *BRCA2* pathogenic germ line mutations were identified in 74 high-risk African American breast cancer or breast-ovarian cancer families, more than in any previous study of blacks.
- Numerous polymorphisms were observed in the *BRCA1* and *BRCA2* genes, and rare variations in *BRCA2*.

- Neither the *BRCA1/2* protein truncating mutations, *BRCA2* rare variations, nor two of the *BRCA2* polymorphisms have been detected in 163 disease-free control subjects.
- The heterozygosity frequency between patients and controls was not statistically significant (two sample T-test) for 10 of the 11 *BRCA2* polymorphisms, indicating that patients and controls are well matched.
- The difference in heterozygosity between patients and African American controls for the intron 10 variation is statistically significant, using a two sample T-test. Therefore, the intron 10 variation may be disease related by controlling splicing or regulation.
- Fifty percent of the *BRCA2* deleterious mutations, 36% of the polymorphisms, and 100% of the rare variations have not been reported previously and may be unique to blacks.
- A 16 base-pair, in-frame deletion at the intron 15/exon 16 boundary of *BRCA2* has been identified. In the African American and Jewish control and patient samples tested, this variant appears to be more common than the Genbank sequence.
- The distribution and frequency of African American *BRCA1* and *BRCA2* mutations from this study and others have been analyzed in terms of family history, ovarian cancers, median age of diagnosis and are similar to those in Caucasians.
- The spectrum of pathogenic *BRCA1/2* mutations in African Americans from this laboratory and others has been analyzed. The P.I. played a major role in writing a review of *BRCA* mutations in African Americans (Olopade et al., 2001)
- The broad spectrum of *BRCA1/2* mutations indicate that genetic testing in African Americans must include the entire coding and flanking non-coding regions of the *BRCA2* gene.

REPORTABLE OUTCOMES

Abstracts and Presentations (*presenter)

Whitfield-Broome, C.* (1998) Inherited breast cancer in African Americans. Segment on Heart and Soul, Black Entertainment Television, October, 1998.

Broome, C.* (1998) Howard U. Cancer Center Symposium on "Innovative Scientific Advances in Breast Cancer Among African-Americans", Washington, D.C. October, 1998. High risk BRCA1 and BRCA2 screening in African American women. Invited presentation.

Broome, C.* (1999) Breast cancer mutations in African Americans. Sigma Xi presentation, Howard University.

Whitfield-Broome C.* (1999) Inheritance of Breast Cancer in African American Women: How Should We Monitor? Presentation, Howard University Women's Health Institute. Health Issues and Concerns of Women of Color: A Call to Action. Washington DC, April, 1999.

Whitfield-Broome, C.*, Kanaan, Y., Kpenu, E., Utley, K., Dunston, G.M., Brody, L.C. (2000) BRCA2 mutations in African Americans. Poster presentation, Era of Hope Department of Defense Breast Cancer Research Program Meeting, Atlanta, GA., June, 2000.

Kanaan, Y.*, Kpenu, E., Utley, K., Brody, L.C., Dunston, G.M., **Whitfield-Broome, C.** (2000a) BRCA2 mutations in African Americans. Poster presentation, Howard University, Graduate School of Arts and Sciences, April, 2000. First place award to Y.K.

Kanaan, Y.*, Kpenu, E., Utley, K., Brody, L.C., Dunston, G.M., **Whitfield-Broome, C.** (2000b) BRCA2 mutations in African Americans. Oral & poster presentation, Howard University

Medical Center Scientific Forum, May, 2000. Second place Roland & Waldean Nickens scientific research award to Y.K.

Kanaan, Y.*, Kpenu, E., Utley, K., Brody, L.C., Dunston, G.M., **Whitfield-Broome, C.** (2000c) BRCA2 mutations in African Americans. Oral & poster presentation, American Society for Microbiology, Washington D.C. branch, student meeting, May, 2000 Third place award to Y.K.

Kanaan, Y.*, Kpenu, E., Utley, K., Brody, L.C., Dunston, G.M., **Whitfield-Broome, C.** (2000d) BRCA2 mutations in African Americans. Oral & poster presentation, National Medical Association, August, 2000. Third place award to Y.K.

Olopade, O.*, Dunston, G., Tainsky, M., Collins, F., **Whitfield-Broome. C.** (2000) Breast cancer genetics. Invited presentation, Summit meeting evaluating research on breast cancer in African American women, Washington D.C., September, 2000.

Whitfield-Broome, C.D.* (2001) Breast Cancer Mutations in African Americans. Invited presentation. Research Centers in Minority Institutions Spring Symposium, Atlanta Georgia, April 26-27, 2001.

Whitfield-Broome, C.*, Kanaan, Y., Kpenu, E., Utley, K., Dunston, G.M., Brody, L.C. (2002) BRCA2 mutations in African Americans. Poster presentation, Era of Hope Department of Defense Breast Cancer Research Program Meeting, Orlando, Fl, September, 2002.

Publications

Whitfield-Broome, C., Dunston, G.M., Brody, L.C. (1999) BRCA2 Mutations in African Americans. American Association for Cancer Research, Philadelphia, April, 1999. Proc. Amer. Assoc. for Cancer Research, 40:269, abstract #1788

Panguluri RCK, Brody LC, Modali R, Utley K, Adams-Campbell L, Day AA, **Whitfield-Broome C,** & Dunston GM. (1999) BRCA1 mutations in African Americans Human Genetics 105:28-31 and on line http://dx.doi.org/10.1007/s004399900085.

Mefford HC, Baumbach L, Panguluri RCK, **Whitfield-Broome C**, Szabo C, Smith S, King MC, Dunston G, Stoppa-Lyonnet D, Arena F. (1999) Evidence for a BRCA1 founder mutation in families of west African ancestry. Am J Hum Genet 65:575-578.

Kanaan, Y.*, Kpenu, E., Utley, K., Brody, L.C., Dunston, G.M., **Whitfield-Broome, C**. (2000e) BRCA2 mutations in African Americans. Poster presentation, American Society of Human Genetics, Philadelphia, October, 2000. Am. J. Hum. Genet. (supplement 2) 67:92 abstract. #455

Olopade, O.I., Fackenthal J.D., Dunston, G., Tainsky, M., Collins, F., **Whitfield-Broome, C**. (2001) Breast cancer genetics in African Americans, (review) submitted.

Kanaan, Y. BRCA2 mutations in African Americans. Howard University Ph.D. Dissertation, December, 2001. [**Broome, C**., advisor]

Kanaan, Y., Kpenu, E., Utley, K., Brody, L.C., Dunston, G.M., **Broome, C. W.** (2002) BRCA2 mutations in African Americans. In preparation.

Development of cell lines

Leukocytes from ten of the high-risk patients have been transformed into permanent cell lines.

New Funding Awarded Based on this Grant

Department of the Army, "Genetics of Breast Cancer in African Americans," principal investigator, $460,048 - direct and indirect costs, 9/01-9/04.

National Cancer Institute, HUCC/Hopkins partnership sub-project: Comparative Gene Expression in African American and Caucasian Breast Cancer, principal investigator, $375,000 –direct costs, 5/01-4/04

Training and Employment

In December 2001, Yasmine Kanaan received her Ph.D. based on her research on this project. She has presented her work at local and national meetings, receiving several awards for her presentations. A manuscript is in preparation (see above). Dr. Kanaan is currently working as a post-doctoral fellow.

CONCLUSIONS

In order to determine the spectrum of germline *BRCA1* and *BRCA2* mutations in African Americans, the entire coding regions and flanking introns have been examined in breast cancer patients from families at high-risk of hereditary breast cancer. Additional breast cancer patients and disease-free control subjects have been recruited; there are now 91 high-risk families, 90 low-risk patients, and 180 controls. Thus, this study represents one of the largest collections of high-risk African Americans whose *BRCA1* and *BRCA2* coding sequences are being completely scanned for mutations. Presently, 51 families have been screened for both *BRCA1* and *BRCA2* mutations; 74 families have been examined for *BRCA2* mutations. The results and their significance are summarized below.

- Two *BRCA1* pathogenic mutations have been detected; one of which is a founder mutation in five families of west African ancestry.
- More deleterious *BRCA2* mutations were identified in this study than in any previous study of blacks.
- Eight pathogenic *BRCA2* mutations have been identified. Two *BRCA2* mutations were observed in male breast cancer patients.
- All of the female breast or ovarian cancer probands with *BRCA2* mutations were diagnosed before the age of 45. Eighty percent of the *BRCA2* female breast cancer patients were diagnosed before age 40 with or without a family history of breast or ovarian cancers.
- Forty percent of our *BRCA1* and *BRCA2* carriers report no family history of breast or ovarian cancers; however, all *BRCA1/2* carrier families exhibited multiple cases of breast/ovarian cancers or other cancers.
- A 16 base-pair, in-frame deletion at the intron 15/exon 16 boundary of *BRCA2* has been identified. In the African American and Jewish control and patient samples tested, this variant appears to be more common than the Genbank sequence.
- Although the numbers for African Americans are low, it appears that the frequency of *BRCA2* mutations is about the same in blacks as in whites.
- Numerous polymorphisms were observed in the *BRCA1* and *BRCA2* genes, and rare variations in *BRCA2.*
- Neither the *BRCA2* protein truncating mutations, rare variations, nor two of the polymorphisms have been detected in 163 disease-free control subjects.

- The heterozygosity frequency between patients and controls was not statistically significant (two sample T-test) for 10 of the 11 *BRCA2* polymorphisms, indicating that patients and controls are well matched.
- The difference in heterozygosity between patients and African American controls for the intron 10 variation is statistically significant, using a two sample T-test. Therefore, the intron 10 variation may be disease related by controlling splicing or regulation.
- A higher frequency of prostate cancer was observed in *BRCA* carriers than in non-carriers. Therefore, multiple cases of breast, ovarian and prostate cancer in a family may be a strong indicator for genetic testing.
- Fifty percent of the *BRCA2* deleterious mutations, 36% of the polymorphisms, and 100% of the rare variations have not been reported previously and may be unique to blacks.
- The importance of this work is for genetic testing and genetic counseling. The broad spectrum of *BRCA1/2* mutations observed by this study and other investigators indicate that genetic testing in African Americans must include the entire coding and flanking non-coding regions of the *BRCA2* gene.
- It is noteworthy that *BRCA* mutations were detected in individuals without a reported history of disease among early onset (≤40 years) breast cancer patients, a male breast cancer patient, and a patient with both breast and ovarian cancers.
- This study supports the importance of early-onset breast cancer (≤ 40), multiple cases of breast cancer with at least one before age 50, ovarian cancer, male breast cancer, and prostate cancer in a family as indicators for genetic testing in African Americans.

Future Directions. DHPLC will be used for *BRCA1/2* mutation detection because it is the most reliable method for *BRCA* mutation detection besides DNA sequencing, which is very expensive (Eng et al., 2001). Rare missense variations, which segregate with disease, will be examined and tested for mutations in exon splice enhancer sequences that lead to exon skipping (Fackenthal et al., 2002)

PERSONNEL

Personnel who received funding on this grant are research Elikem Kpenu, research assistant; Kim Utley, subject recruiter; Yasmine Kanaan, graduate student, Angela Jackson, graduate student; Marina Ganpat, postdoctoral research associate; Meseret Ashenafi, postdoctoral research associate; Desta Beyene, postdoctoral research associate.

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BRCA2 MUTATIONS IN AFRICAN AMERICANS

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In order to identify BRCA2 mutations in African Americans, 74 breast cancer patients from families at high risk of hereditary breast cancer were studied. The entire coding regions and flanking introns of BRCA2 have been screened for germline mutations by single stranded conformational polymorphism, protein truncation test, or denaturing high performance liquid chromatography followed by DNA sequencing. Eight protein truncating, pathogenic mutations have been detected in female and male patients but not in 163 disease-free control subjects. All of the pathogenic mutations delete 18-84% of the protein including important functional domains such as the Rad51 binding site and nuclear localization sequence. Four (1991delATAA, 1993delAA, 2001delTTAT, 8643delAT) of the eight pathogenic mutations observed in African Americans have not been previously described and may be unique to this group. The other four pathogenic mutations (1882delT, 2816insA, 4075delGT, 4088delA) detected in African Americans have been previously reported. Eighty percent of the pathogenic mutations in female breast cancer patients were identified in women diagnosed with breast cancer under the age of 40 with or without a family history of the disease. Six rare variations, not previously detected, were identified in five breast cancer patients but not in 163 disease-free control subjects. Of eleven polymorphisms identified in high-risk African American breast cancer patients, four may be unique to African Americans. The intron 10 polymorphism observed in patients was not detected in 163 disease-free African American control subjects; this difference is statistically significant. Since many different pathogenic mutations and variants of unknown significance are observed in African Americans, BRCA2 genetic testing in high-risk African American families must include the entire coding and flanking non-coding regions of the gene. This study supports the importance of early-onset breast cancer (< 40), male breast cancer, multiple cases of breast cancer with at least one before age 50, ovarian cancer, and prostate cancer in a family as indicators for BRCA genetic testing in African Americans.

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Abstract submitted for the Era of Hope 2002 Department of Defense Breast Cancer Research Program Meeting – Orlando,Florida, September 25-282002

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Announces

The Final Examination of

Ms. Yasmine M. Kanaan

In defense of the Dissertation:

"*BRCA2* Mutations in African Americans"

THE DOCTOR OF PHILOSOPHY DEGREE

Department of Microbiology

November 27, 2001

10:00 a.m.

Room 205
Graduate School
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Washington, D C 20059

DISSERTATION ABSTRACT - Yasmine Kanaan

Since the identification of the *BRCA2* breast-ovarian cancer susceptibility gene, mutation analyses have been carried out in various populations revealing ethnic-associated mutations. In order to identify *BRCA2* mutations in African Americans, 74 breast cancer patients from families at high risk of hereditary breast cancer were studied. The entire coding regions and flanking introns of *BRCA2* have been screened for germline mutations by single stranded conformational polymorphism, protein truncation test, or denaturing high performance liquid chromatography followed by DNA sequencing. Eight protein truncating, pathogenic mutations have been detected in the patients but not in 163 disease-free control subjects. All of the pathogenic mutations delete 18-84% of the protein including important functional domains such as Rad51 binding site and nuclear localization sequence. Four (1991delATAA, 1993delAA, 2001delTTAT, 8643delAT) of the eight pathogenic mutations observed in African Americans have not been previously described. Therefore, half of the pathogenic mutations observed are unique to African Americans. The other four pathogenic mutations (1882delT, 2816insA, 4075delGT, 4088delA) detected in African Americans have been previously reported. Two of the pathogenic mutations, 1991delATAA and 2816insA, were detected in male breast cancer patients, consistent with previous studies showing an association of *BRCA2* with male breast cancer. Only one of the eight pathogenic mutations, 2816insA, is recurrent in other African American families. Eighty percent of the pathogenic mutations in female breast cancer patients were identified in women diagnosed with breast cancer under the age of 40 with or without a family history of the disease.

Six rare variations, which occur at a frequency of less than 1 in 100 chromosomes, were identified in five breast cancer patients but not in 163 disease-free control subjects. None of these rare variations, which occur in the non-coding regions or result in a silent amino acid change, have been previously reported. Eleven polymorphisms, which occur at a frequency of greater than 1 in 100 chromosomes, were identified in high-risk African American breast cancer patients. Four of these polymorphisms have not been reported and may be unique to African Americans. The intron 10 polymorphism was not detected in 163 disease-free African American control subjects; this difference is statically significant. Considering our work and that of others, many different pathogenic mutations and many variants of unknown significance are observed in African Americans; therefore, *BRCA2* genetic testing in high-risk African American families must include the entire coding and flanking non-coding regions of the gene. Although the numbers for African Americans are low, it appears that the frequency of *BRCA2* mutations is about the same in blacks as in whites. Therefore, the same criteria for genetic testing in Caucasians are recommended for African Americans. Since *BRCA* mutations result in a predisposition to prostate cancer, multiple cases of breast, ovarian, and prostate cancer in a family may signal a reason for genetic testing.

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Suppression of Androgen Receptor Expression as a Therapeutic Objective in Prostate Cancer.
Dr. Wayne Harris

Functional androgen receptor expression persists in most cases of hormone-refractory prostate cancer. Current evidence supports the hypothesis that ligand-independent activation of the androgen receptor is an important mechanism of progression from hormone-responsive to hormone-refractory disease. Common therapies for the initial treatment of metastatic prostate cancer are directed toward the removal of androgen by surgical or medical castration. Another common strategy involves the use of oral nonsteroidal antiandrogens. These agents prevent nuclear uptake of androgen by competitively interfering with the binding of androgens to the androgen receptor. Unfortunately, neither of the current approaches prevents ligand-independent activation of the androgen receptor.

We propose androgen receptor suppression therapy as a completely new approach to the prevention and treatment of prostate cancer with the potential for broad clinical application. In the absence of expressed androgen receptor, disease progression based on ligand-independent activation cannot occur. Of course, there are potential risks to this approach. There may be vital cellular functions in normal cells that are mediated by the androgen receptor that do not involve androgens. For this reason, a detailed understanding of the mechanisms that control androgen receptor expression and degradation is vital to developing tissue-specific androgen receptor suppression therapies.

Breast Cancer Mutations in African Americans.
Dr. Carolyn D. Whitfield-Broome

Hereditary breast cancer accounts for 3-8% of all breast cancer. Over 80% of inherited breast cancer is due to mutations in the breast cancer predisposing genes *BRCA1* and *BRCA2*. In one of the largest studies of high-risk African American families, our group has identified pathogenic, protein-truncating mutations in *BRCA1* and *BRCA2*, including a *BRCA1* founder mutation of West African origin. *BRCA1* mutations were detected in a patient with breast and ovarian cancer, and in a family with five cases of breast cancer. *BRCA2* mutations were observed in families with multiple cases of breast cancer or breast and ovarian cancers; in women diagnosed with breast cancer before age 40 having a family history of breast or ovarian cancers or other cancers; and in male breast cancer patients. Our data combined with that of other investigators reveal that 58% of the *BRCA1* and *BRCA2* mutations carried by African Americans and Africans are unique to this group. Less than 7% of the *BRCA1* and *BRCA2* mutations are observed in more than two families. Genetic testing for *BRCA* mutations is most beneficial in families with 4 or more cases of breast or ovarian cancers, and in families with at least one breast cancer diagnosed before age 50 or ovarian cancer diagnosed at any age and one first degree (mother, sister, daughter) relative with either breast or ovarian cancer. Since African Americans have a unique mutation spectrum with few recurrent mutations, genetic testing for *BRCA1* and *BRCA2* mutations should involve the entire coding and flanking sequences. Supported by U.S. Army Medical Research and Materiel Command, DAMD17-98-1-8106, Susan Komen Foundation, and NIH G12 RR03048.

I. Title Page

Breast Cancer Genetics in African Americans

Running Title: African American Breast Cancer Genetics

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Total number of text pages: 7
Total number of Tables: 3

II. Precis for table of contents

BRCA1 and *BRCA2* mutations have been reported in a number of African American and African families but only a few mutations are recurrent and suggestive of a common ancestry. Therefore, genetic testing in the clinical arena should involve searching for mutations in the entire coding region and flanking sequences of both genes.

III. Abstract

BACKGROUND. An overview of the state of genetic testing for *BRCA1* and *BRCA2* genes was presented at the African American Breast Cancer Summit.
METHODS. An exhaustive literature search was performed using PubMed, abstracts published from meetings of the American Association for Cancer Research, the American Society of Human Genetics, and the American Society of Clinical Oncology. The Breast Cancer Information Core was also searched for information regarding sequence variants in which the ethnicity of the individual tested was known.
RESULTS. Of the 26 distinct *BRCA1* pathogenic mutations (protein truncating, disease-associated missense and splice variants) detected in Africans or African Americans, 15 (58%) are unique to this group. In addition, 18 deleterious *BRCA2* mutations have been identified and 10 (56%) of these are unique to the group. Only two pathogenic *BRCA1* mutations (943ins10 and M1775R) have been detected in more than two unrelated families. However, seven additional *BRCA1* or *BRCA2* deleterious mutations have been reported in at least two unrelated families. Three of these recurrent *BRCA1* mutations (943ins10, 1832del5 and 5296del4) have been characterized by haplotype analysis and each likely arose from a common ancestor, including one ancestor that could be traced back to Ivory Coast in West Africa. Although the numbers of families with deleterious mutations are small, the probability of finding a mutation in *BRCA1* or *BRCA2* is invariably dependent on the age of onset and number of breast and/or ovarian cancer cases in the family. In families with two or more breast and/or ovarian cancers where at least one breast cancer was diagnosed before age 50 years, the proportion with *BRCA1* or *BRCA2* pathogenic mutations is minimally 18% in breast cancer only families and 38% in families with breast and ovarian cancers. While the psychosocial implications of genetic testing for African Americans have not been well studied, there is a suggestion that high risk African Americans underestimate their risks of breast cancer.
CONCLUSIONS. African American and Africans may have a unique mutation spectrum in *BRCA1* and *BRCA2* genes but recurrent mutations are likely to be widely dispersed and therefore not readily identifiable in these populations. More resources should be available for establishing a large cohort of African American and African families, defining prevalence and the mutation spectrum in *BRCA1* and *BRCA2* genes and examining gene-gene and gene-environment interactions that contribute to breast cancer in these families. Access to genetic counseling and testing in a culturally sensitive research setting must remain a high priority before genetic testing could be disseminated in the community.

IV. Key Words

African American, African, Breast, Cancer, Genetics, Testing, Prophylactic Surgery, *BRCA1*, *BRCA2*, Genetic Counseling

V. Text

Breast cancer mortality is high among young African American women who contract the disease. A clear understanding of associated risk factors will likely lead to the development of novel interventions to prevent breast cancer and drastically reduce mortality from the disease. One of the most promising areas of research is the identification of genes, gene-environment or gene-gene interactions that lead to increased breast cancer risk among young African American women. Estimates from population based studies suggest that about 3-8% of breast cancers are explained by germline mutations in highly penetrant susceptibility genes such as *BRCA1* and *BRCA2*. Genetic testing for *BRCA1* and *BRCA2* has become a reality, moving from the research setting into clinical practice. To ensure equity and justice in the clinical use of genetic testing, an understanding of the unique needs and concerns of different populations is necessary. This manuscript reviews the state of the technology for African American women and identifies opportunities for research into the genetic bases of breast cancer in the group.

MATERIALS AND METHODS

Information was gathered from searching (through February 2001) PubMed, the Breast Cancer Information Core, abstracts published from meetings of the American Association for Cancer Research, the American Society of Human Genetics, and the American Society of Clinical Oncology.

RESULTS AND DISCUSSION

Parallels between BRCA-related cancers and African American breast tumors

There appear to be striking similarities between BRCA1-related breast cancers and breast cancers that occur in young African American women. For example, *BRCA1*-associated breast cancers occur at an earlier average age (44 years) than sporadic breast cancers [1,5], and it has been observed that African American patients have a greater breast cancer incidence between 30-49 years than whites [3]. Additionally, compared to non-carriers, *BRCA1*-associated breast cancers are characterized by higher than expected frequencies of medullary or atypical medullary carcinoma, poorly differentiated (high tumor grade), aneuploidy, high S-phase fraction, hormone receptor negativity, and p53 mutation [4-8]. In contrast to *BRCA1*, *BRCA2* tumors are more differentiated with more tubular and lobular features and a higher rate of hormone receptor positivity [5,6,7]. Tumors in young African-American women are also more likely to be poorly differentiated, hormone receptor negative, and to exhibit high nuclear atypia, and higher S-phase [9,10]. A significant proportion of breast cancers in African American women exhibit medullary or atypical medullary features[10]. These facts suggest a strong genetic contribution to breast cancer in African-American women but limited data are available from this population to evaluate this possibility.

Deleterious *BRCA* mutations among African Americans

***BRCA1* Mutations.** Hereditary breast cancer accounts for 3-8% of all breast cancer [11,12]. The highly penetrant, breast cancer predisposing genes, *BRCA1* and *BRCA2* contribute to about 80% of hereditary breast cancer [13-16]. Recently, an additional breast cancer susceptibility locus has been reported [17]. *BRCA1* and *BRCA2* function in double-strand DNA repair, homologous recombination, transcription regulation, embryonic growth, and cell cycle regulation [18]. Deleterious *BRCA* mutations, such as protein truncating, disease-associated missense, and

splicing mutations, abolish the function of the BRCA proteins. Germline *BRCA* mutations are usually hetereozygous and cancer occurs through somatic loss/inactivation of the wild type allele. Without a functional test or segregation with disease in a large affected family, it is difficult to determine if missense and noncoding variations cause disease.

Most genetic epidemiology studies have focused on Caucasian women with a strong family history of breast and/or ovarian cancer although a few African American families were included in the earliest studies. Pathogenic *BRCA1* amino acid substitutions (missense mutations M1775R, C64G), which segregate with the disease in large African American families and occur in functionally important regions of the protein were first described in 1994 [13,19-21] (Table 1). *BRCA1* protein truncating, frameshift mutations have now been reported by multiple groups [22-24] (Table 1). Three frameshift mutations were identified by Arena et al. [22] in Florida African Americans with a strong family history of the disease, including a 10 base pair duplication (943ins10). Stoppa-Lyonnet et al. detected the same 10 base pair duplication (943ins10) in one family who immigrated from the Ivory Coast. Gao et al., [23] identified five protein truncating *BRCA1* mutations in nine high-risk families (56%). These *BRCA1* mutations were identified in early-onset breast and/or ovarian cancer families with average ages at diagnosis below 40 years. Four of the five *BRCA1* mutations were also identified in families with three or more cases of breast and/or ovarian cancer. In a study of 85 African American breast cancer patients, one additional *BRCA1* frameshift mutation (1625del5) was identified by Gao et al. [25].

Ganguly et al. (1998) reported two deleterious *BRCA1* mutations among 10 African American patients with a family history of breast cancer [26]. Panguluri et al. (1999) identified 2 deleterious *BRCA1* mutations, 943ins10 and 3450del4, among 45 high-risk African American families treated at Howard University Hospital and selected for family history, early age of onset, breast and ovarian cancer, bilateral breast cancer, and male breast cancer [27]. Shen et al. (2000) examined exons 2, 5, 11, 16, and 20 of the *BRCA1* gene in 54 African American breast cancer patients, not selected for family history or age, and found one novel frameshift mutation [28]. Other mutations/variations in *BRCA1* have been reported to the Breast Cancer Information Core (BIC) [29] by Myriad Genetic Laboratories and other investigators (Table 1).

In a population based case-control study in North Carolina, white women exhibited a higher frequency of *BRCA1* mutations than black women [30]. No disease-related *BRCA1* mutations were identified in 88 black women with breast cancer; three pathogenic *BRCA1* mutations were identified in 120 white women with breast cancer. Family history and age of onset were not delineated by race for the cases. A polymorphism in the 3' untranslated region of *BRCA1,* which is in linkage disequilibrium with a polymorphism in intron 22, was detected at a significantly higher frequency in African American cases than in black controls.

Combining the data of Olopade and colleagues as well as Whitfield-Broome and colleagues, the following summarizes our observations in African American families with at least two cases of female breast cancer or breast and ovarian cancers among first degree relatives [25,27]. The proportion of families with *BRCA1* mutations in breast only families was 10% (4/39). In families with both ovarian and breast cancers, the proportion with *BRCA1* mutations was 38% (3/8). In families with at least one breast cancer diagnosed before age 50 and no ovarian cancer, the proportion with *BRCA1* mutations was 16% (4/25). In families with at least one breast cancer diagnosed <50 years and ovarian cancer diagnosed at any age, the proportion with *BRCA1* mutations was 43% (3/7). The median age of diagnosis in *BRCA1* carriers was 33.7 years (n=7; range 23-40), significantly different ($p < 0.001$, t test) from the median age of 49.3 in non-carriers

(n=35, age range 29-70) [25,32]. Comparable observations have been made in Caucasian populations [1,15,16,31]. Couch et al. reported that 7% (9/124) of multiple case Caucasian families with breast cancer and no ovarian cancer carried *BRCA1* mutations. However 40% (18/45) of families with breast and ovarian cancer exhibited *BRCA1* mutations. There was a significantly increased probability of identifying *BRCA1* mutations when family members are diagnosed with breast cancer at an age <55 years [1]. In primarily white women with breast cancer diagnosed before age 50 or ovarian cancer diagnosed at any age and one first degree or second degree relative with either diagnosis, Frank et al. observed *BRCA1* mutations at a similar frequency of 18% (22/121) in families with breast cancer only. *BRCA1* mutations were detected in 35% (41/117) of families with breast and ovarian cancers. [31].

Of the 26 distinct *BRCA1* pathogenic mutations detected in African Americans or Africans, 58% (15/26) are unique to this group (Table 1). Twenty-three percent (6/26) of the pathogenic mutations have been detected in more than one family of African ancestry. Only two (8%) deleterious mutations, M1775R and 943ins10, are carried by more than two families. Therefore, African Americans exhibit a unique broad spectrum of deleterious *BRCA1* mutations and variations [25-27].

***BRCA2* Mutations.** The *BRCA2* gene, which is twice as large as *BRCA1*, has been less studied in African Americans than *BRCA1*. A recurrent *BRCA2* frameshift mutation, 2816insA, has been identified in African American families with breast and ovarian cancers and male breast cancer [25,33,34]. Ganguly et al. (1998) reported three different disease-related *BRCA2* mutations among 10 African American patients with a family history of breast cancer [26] (Table 2). Four distinct disease-related *BRCA2* mutations in African Americans have also been reported by Myriad [29].

In a study of 74 high-risk African American breast cancer patients, treated at Howard University Hospital and selected for family history as described above, Whitfield-Broome and colleagues identified 8/74 (11%) pathogenic, *BRCA2* frameshift mutations after examination of the entire coding and flanking sequences (Table 2) [32,34]. Numerous polymorphisms and non-coding variants were also observed in the *BRCA2* gene. Fifty percent of the pathogenic mutations were novel and possibly unique to African Americans and one-half were observed in women below the age of 40, with a family history of breast or ovarian cancers or other cancers. Two pathogenic mutation carriers were males.

In the population based case-control study in North Carolina, white women exhibited a 3 times higher frequency of *BRCA2* mutations than black women [35]. One disease-related *BRCA2* mutation was identified in 88 black women with breast cancer. In a world-wide study of 71 breast cancer families and 95 controls, Wagner et al. (1999) did not identify any deleterious *BRCA2* mutations in African American or African families [36]. Consistent with the greater genetic diversity observed in people of African ancestry, a higher frequency of sequence variations was found in Africans than in other world-wide populations.

By combining the *BRCA2* data of Olopade and Whitfield-Broome and their associates [25,32] the following is observed. The proportion of *BRCA2* mutations in breast cancer only families was 6% (3/54) and 33% (4/12) in families with breast and ovarian cancers although the numbers are too small to make any generalization. Frank et al. also detected *BRCA2* mutations at a frequency of 11% (13/121) in families with breast cancer only and at a lower frequency of 15% (18/117) in families with breast and ovarian cancers. [31] Among Caucasian families, *BRCA1* mutations are far more common than *BRCA2* mutations in breast and ovarian families; while

BRCA2 mutations account for the same or higher proportions in breast cancer only families.[15,16,31].

In our African American cohort, the median age of diagnosis of breast cancer among *BRCA2* carriers was 44.4 years (n=7, age range 34-58), not significantly different from 48.3 years in non-carriers (n=51, age range 29-70) [25,32]. Among Caucasian families with 4 or more cases of breast cancer or breast and ovarian cancers, the median age of diagnosis of female breast cancer among *BRCA2* carriers was 45.1 years, similar to that observed in African Americans [16]. African American *BRCA2* mutation carriers develop cancer at a significantly later age of onset than *BRCA1* mutation carriers ($p < 0.05$), as has been described for Caucasian populations [15,16,31].

Of the 18 distinct pathogenic *BRCA2* mutations detected in families of African ancestry, 56% (10/18) are novel and probably unique to this group (Table 2). Seventeen percent (3/18) of the pathogenic mutations have been detected in more than one African American or African family. No *BRCA2* mutations were carried by more than two families of African ancestry. This distribution of African American *BRCA2* mutations is very similar to that for *BRCA1*. Likewise, most of the distinct *BRCA1* and *BRCA2* mutations reported in the BIC (frequency graphs) occur in only a small number of families [29]. Numerous *BRCA1* and *BRCA2* polymorphisms, missense and noncoding variants are observed in African Americans (Tables 1 and 2). Large deletions in *BRCA1* and *BRCA2* would not be identified by the techniques used.

Breast cancer and *BRCA* mutations among Africans

In populations indigenous to the African tropics, breast cancer has been considered to be a rare disease, predominantly afflicting young women [37]. However, the International Agency of Research on Cancer Bulletins and surveys in seven African countries has shown that breast cancer incidence increased from 15.3 per 100, 000 in 1976 to 33.6 per 100,000 in 1998 [37]. Incident female breast cancer rates for whites and blacks in the United States are 113.2 and 99.3 per 100,000 respectively [38]. The rising incidence in Africa has been attributed to increased reporting and the adoption of a western lifestyle in urban cities. In a recent review of breast cancer cases from the University of Ibadan, Nigeria, the average age at diagnosis was 42.6 years, 10-15 years younger than in whites [39]. The young average age at diagnosis could be partly explained by the low mean age of the general African population. However, multiple studies in the United States (U.S) have also documented a higher breast cancer incidence and death rate in pre-menopausal black women compared to non-Hispanic whites (http://www-seer.ims.nci.nih.gov). Thus, it is likely that the shared genetic background of Africans and U.S blacks contributes to the greater susceptibility to early onset breast cancer in both groups but this has not been carefully examined. In the first study of it's kind, the entire coding regions as well as the intron/exon boundaries of *BRCA1* and *BRCA2* have been examined in 70 African breast cancer patients under 40 years of age [40]. These patients were ascertained at the University of Ibadan College of Medicine, Nigeria and were not selected for a family history of breast cancer. In fact, the majority of patients in the cohort reported no family history of breast cancer, indicating that the results presented below would have been missed had the subjects been selected for family history. In this cohort, two *BRCA1* truncating mutations, four *BRCA1* missense variations, one *BRCA2* truncating mutation and nine different *BRCA2* missense variations were identified (Tables 1, 2). The truncating *BRCA1* mutation Q1090X has never been described previously and was not seen outside of one family identified in this cohort. The

1742insG mutation is also unique to this cohort. The *BRCA1* amino acid substitution alleles, however, have all been described in other populations: alleles E1038G and K1183R have both been described as benign polymorphisms, and I379M and K820E have both been described as unclassified variants [29]. The *BRCA2* truncating mutation 3034del4 has previously been described as a mutational hotspot [41]. The *BRCA2* missense variation N1880R has never been reported previously. The *BRCA2* alleles G3212R, A248T, N987I, and L929S have all been reported independently and are listed as unclassified variants [29].

Founder mutations among families of African ancestry

When the same mutation is found in multiple unrelated families, this may be due to ancestry from a small isolated group of founders or to independent mutational events. A common haplotype among unrelated families around the gene of interest is evidence for a founder effect. The length of the common haplotype is inversely related to the age of the mutation. The *BRCA1* 943ins10 mutation was associated with a single haplotype in 5 families from Ivory Coast, Washington DC, Florida, South Carolina, and the Bahamas [42]. The length of the common haplotype is about the same as the Ashkenazi Jewish founder mutation, 185delAG, which has been estimated to be 760 years old [43]. Therefore, the *BRCA1* 943ins10 mutation appears to be an ancient founder mutation of West African origin. A common haplotype was reported for two African American families with the *BRCA1* 5296del4 mutation and for two African American families with the *BRCA1* 1832del5 [23]. As shown in Tables 1 and 2, *BRCA* mutations detected in African Americans or Africans have also been reported multiple times in families with and without African ancestry, but haplotype analysis is needed to determine if they represent founder mutations unique to African Americans. Due to the higher level of variations/polymorphisms among African Americans compared to Caucasians, wide geographic origins in Africa, and genetic admixture, recurrent mutations are likely to be more widely dispersed in the African diaspora and therefore not readily identifiable in this population [25-27,32]. Therefore, genetic testing in African Americans must include complete sequencing of both *BRCA1* and *BRCA2* genes.

Familial cancer syndromes that are prevalent among African Americans

Little information exists about other familial cancer syndromes unique to African-Americans but two African-American families with Cowden's syndrome have been reported [44]. The same germline p53 coding mutation and haplotype were detected in two Li-Fraumeni African American families, one of which exhibited primarily breast and ovarian cancer [45].

Identification of African American families that may benefit from genetic testing

Pre- and post- genetic counseling is extremely important for genetic testing. Genetic counseling translates basic scientific advances into a practical and understandable form of information for the patient. It involves the collection of medical and family information, recognition of familial syndromes based on pedigree analysis, calculation of risk estimates, and effective communication of risk status at a level that the patient can understand. The National Society of Genetic Counselors has developed guidelines that counselors should follow [46,47]. These include respect for autonomy and privacy of the individual, the need for confidentiality and informed consent, and the provision of information to the patient in a nondirective manner [48,49]. Most importantly, the patient should be educated about cancer prevention practices. As cancer risk assessment moves from the research setting into clinical practice, genetic counseling and patient education must be an integral part of such programs. To ensure equity and justice in

the clinical use of genetic testing, an understanding of the unique needs and concerns of different populations is necessary.

White women from socioeconomically advantaged circumstances comprise the majority of clients who use genetic counseling and testing options even when covered by third party payers [50,51]. It has long been recognized that minority and economically disadvantaged patients do not participate in clinical trials [52]. This situation has been attributed to a number of reasons including: ignorance of the availability, fear and distrust, perception of cost, access and transportation, manner of information presentation, lack of valid, culturally sensitive questionnaires and language of consent forms [53-55]. Although data are limited, anecdotal reports suggest that minorities are also less likely to use cancer genetics services unless a major outreach effort is directed towards their inclusion. The University of Chicago Cancer Risk Clinic has made a major commitment to provide avenues for women of all socioeconomic background to take advantage of recent advances in cancer genetics, including genetic testing, pre- and post-counseling [23,25,31,56]. In a recent report on the African American Hereditary Prostate Cancer (AAHPC) Study, an ongoing multicenter genetic linkage study organized by investigators at Howard University and the National Human Genome Research Institute in collaboration with a consortium of predominantly African American urologists, Royal et al. found that physician referral and tumor registries were by far the most productive recruitment mechanisms[69]. The challenges and successes of the recruitment experience described in the first phase of the AAHPC study should serve to inform future efforts to involve this population in similar studies.

Based on the fact that *BRCA* mutations are rare in the general population, it is recommended that genetic testing be recommended in the context of a comprehensive cancer risk assessment. There is no reason to suspect that the expression of *BRCA* mutations in African American families with breast and ovarian cancers are different from Caucasian families. Therefore, general guidelines that have been published by other professional groups should be adhered to. The American Society for Clinical Oncology recommends that genetic testing be considered when there is at least a 10% probability of detecting a deleterious mutation. Thus, genetic counseling and testing should probably be offered to individual patients with early onset disease (under age 35 years), and multicase families in which at least one breast cancer was diagnosed before age 50 or ovarian cancer diagnosed at any age. Families with at least one male breast cancer are also candidates for testing. Genetic testing could provide valuable information because *BRCA* carriers with breast cancer have a 10-fold elevated risk of developing ovarian cancer and increased risk of contralateral breast cancer, compared to non-carriers [31]. Recently, bilateral prophylactic oophorectomy in *BRCA* carriers has been reported to reduce the risk of breast cancer and ovarian cancer [57,58]. Hence, we should intensify efforts to integrate genetic counseling and testing into the clinical care of young African American women already affected by cancer. Identification of deleterious mutations in index breast cancer cases could provide the link to other at risk family members. Access to genetic testing may help minority women from such high risk families develop better strategies to reduce their risk of dying from breast or ovarian cancer. However the complexities of genetic testing including the unresolved psychosocial issues warrant that more research be done.

Sensitivity of genetic counselors to cultural/ethnic issues

The University of Chicago Cancer Risk Clinic conducted 3 focus group sessions to evaluate the informational needs of African Americans participating in genetic testing trials. Most participants felt African American culture speaks to the situation of genetic testing with a

unique voice -- that African Americans' collective history brings up a set of issues related to cancer prevention, treatment and medical research in general, that is distinct from Euro-American issues. At the same time, participants acknowledged the universality of the human experience with cancer, and the desire to be treated like everyone else.

Conclusions and Recommendations from the focus group studies include:

- Provide a forum where African Americans with family histories of cancer can discuss issues related to cancer.
- Use a local spokesperson or community leader who is African American, a cancer survivor, or has participated in genetic testing to speak at community centers.
- Develop a media campaign using a celebrity spokesperson who is African American, has had cancer, or has a family history of cancer.
- Provide a toll-free or local phone number for people to call with any questions about genetic testing.
- Use community centers and high traffic areas such as churches, public transportation, schools, park district centers, and libraries to distribute written information and post the toll-free or local phone number.

An intensive outreach program focused on cancer control through genetics could improve awareness of genetics in the African American community. Compared to Caucasian women, African American women had lower levels of knowledge and had more positive attitudes about the benefits of genetic testing [59]. There were no significant ethnic differences in attitudes about the limitations and risks of testing, however, income was negatively associated with this outcome. Ethnic differences in knowledge and attitudes about genetic testing for breast-ovarian cancer risk may be attributable to differences in exposure to genetic information and referral by health care providers.

Little information exists about rates or predictors of test use among African Americans. In a few studies that have examined rates of test use among individuals from high-risk families who have self-referred for genetic counseling/testing, it appears that spiritual faith and psychological factors influenced testing decisions. Lerman and her colleagues have recently published their findings in 290 (including African American) women with familial breast cancer who were offered genetic counseling and testing for alterations in the *BRCA1* and *BRCA2* genes [60]. Baseline levels of spiritual faith, cancer-specific distress, perceived risk, and demographic factors were examined to identify independent predictors of whether participants received versus decline testing. Among women who perceived themselves to be at low risk of developing breast cancer again, those with higher levels of spiritual faith were significantly less likely to be tested, compared with those with lower levels of faith (OR, 0.2; 95% CIs, 0.1 and 0.5). However, among women with high levels of perceived risk, rates of test use were high, regardless of levels of spiritual faith (OR, 1.2; 95% CIs, 0.4 and 3.0). These results highlight the role that spirituality may play in the decision-making process about genetic testing. Unfortunately, in our experience, we have found that African American women generally underestimate their risk and this correlated with higher levels of spiritual faith. In fact, despite extensive genetic counseling, cancer risk perception among African-American women remains closely associated with personal experiences [61]. Consideration of these factors may be important in effectively designing risk assessment and education programs for minority women.

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TABLE 1

***BRCA1* Mutations in African American and African Breast Cancer Patients**

BRCA1 mutations	Effect	No. Af Ances Fam	No. non-Af Ances Fam	References
Protein Truncating				
155del4	FS	1 Af Am	0	62
943ins10	FS	7 Af Am, 1 Af	2	22,24,27,29,42,63,64
1625del5	FS	1 Af Am	0	25
1742insG	FS	1 Af	0	40
1832del5	FS	2 Af Am	0	23
E673X	N	1 Af Am	0	29
2418delA	FS	2 Af Am	0	29
3331insG	FS	1 Af Am	0	28
3450del4	FS	1 Af Am	9	27,29
Q1090X	N	1 Af	0	40
3875delGTCT	FS	1 Af Am	35	29,65
3883insA	FS	1 Af Am	0	23
3888delGA	FS	1 Af Am	0	22
K1290X	N	1 Af Am	1	29
4160delAG	FS	1 Af Am	0	22
Y1463X	N	1 Af Am	2	29
4730insG	FS	1 Af Am	0	29
4794insA	FS	1 Af Am	0	26
5296del4	FS	2 Af Am	9	23,29
Disease Associated Missense Mutations				
C61G	MS	2 Af Am	65	33
C64G	MS	1 Af Am	2	20,29
W1718C	MS	1 Af Am	1	29,66
M1775R	MS	5 Af Am	7	13,19,29,33

Table 1 (continued)

BRCA1 mutations	Effect	No. Af Ances Fam	No. non-Af Ances Fam	References
Splicing Mutations				
IVS4-1G/T	S	1 Af Am	9	26,29
IVS13+1G/A	S	1 Af Am	0	29
IVS22+5G/T	S	1Af Am	0	67
Unclassified Variants and Polymorphisms				
K38K (G233A)	P	2 Af Am	2	29,30
S186Y	UV	1 Af Am	2	25,29
M297I	UV	1 Af Am	0	29
R315G	UV	1 Af Am	0	29
K355R	UV	1Af Am	0	29,68
Q356R (A1186G)	P	4 Af Am	90	29,68
I379M	UV	3 Af Am, 1Af	4	29,30,40
A521T	UV	1Af Am	0	29
S616delS	IFD	1 Af Am	1	29
N723D	UV	1 Af Am	5	29
L771L (T2430C)	P	34 Af Am	159	29,30
K820E	UV	9 Af Am, 1 Af	13	29,40
P871L (C2731T)	P	1 Af Am	161	29,68
P938P (A2933G)	P	2Af Am	0	30
E1038G	P	34 Af Am	203	29,30
S1040N	P	2 Af Am	21	29,30
S1140G (A3537G)	P	14 Af Am	19	23,25,27-30
K1183R (A3667G)	P	36 Af Am, 1 Af	186	27-30,40
Q1200H	UV	2 Af Am	0	29,30
L1260L (T4932C)	P	4 Af Am	0	30,68

Table 1 (continued)

BRCA1 mutations	Effect	No. Af Ances Fam	No. non-Af Ances Fam	References
S1297F (C4009T)	UV	1 Af Am	0	28
L1564P	UV	2 Af Am	1	27,29
L1605L (T4932C)	P	2 Af Am	0	30
Q1785H	UV	1 Af Am	0	27
E1794D	UV	1 Af Am	0	27
IVS1-10T/C	UV	2 Af Am	0	29
IVS8-58delT	UV	36 Af Am	99	29,30
IVS12+12delGT	P	2 Af Am	0	29,30
IVS16-?68G/A	P	34 Af Am	95	29,30
IVS18+? A/G	P	34 Af Am	79	29,30
IVS19+85delT	UV	1 Af Am	0	29
IVS20+59ins12	P	2 Af Am	33	29,30
IVS22+7 T/C	UV	1 Af Am	0	27
IVS22+8 T/A	UV	1 Af Am	0	27
IVS22+8 T/C	P	7 Af Am	8	29,30,65
IVS22+67 T/C	P	5 Af Am	0	27,65
IVS22+78 C/A	UV	2 Af Am	0	27
IVS23-10C/A	UV	1 Af Am	6	29
C5817G (3'UTR)	P	18 Af Am	4	29,30

Af Am, African American; Af, African; Af Ances Fam, African ancestry families; non-Af Ances, not of African Ancestry; FS, frameshift (identified by nucleotide, nt); N, nonsense (codon); MS, missense (codon); IVS, intervening sequence (intron no., nt); S, splicing (nt); P, polymorphism (>1 out of 100 chromosomes in breast cancer cases or controls); UV, unclassified variant (amino acid substitutions are identified by codon first and by nt in parentheses); IFD, in-frame deletion (codon); UTR, untranstlated region.

TABLE 2

***BRCA2* Mutations in African American and African Breast Cancer Patients**

BRCA2 mutations	Effect	No. Af Ances Fam	No. non-Af Ances Fam	References
Protein Truncating Mutations				
1536del4	FS	1 Af Am	0	25
1882 del T	FS	1 Af Am	2	29,32
1991delATAA	FS	1 Af Am	0	32,34
1993delAA	FS	1 Af Am	0	32
2001delTTAT	FS	1 Af Am	0	34
2816insA	FS	2 Af Am	5	25,29,32,34
3034del4 (3036del4)	FS	1 Af	35	29,40
4075delGT	FS	1 Af Am	16	29,32
4088delA	FS	1 Af Am	1	29,32
6696delTC	FS	1 Af Am	2	25,29
Q2342X	N	1 Af Am	0	29
7436del4	FS	1 Af Am	0	26
7795delCT	FS	2 Af Am	0	25,29
7907delTT	FS	1 Af Am	0	26
8643 delAT	FS	1 Af Am	0	32
9481insA	FS	2 Af Am	2	29
R3128X	N	1 Af Am	7	29
Splicing Mutations				
IVS13-2A/G	S	1 Af Am	0	26
Unclassified Variants and Polymorphisms				
P46S	UV	1 Af Am	0	29
P59A	UV	1 Af Am	0	29

TABLE 2 (continued)

BRCA2 mutations	Effect	No. Af Ances Fam	No. non-Af Ances Fam	References
N108H	P*	1 Af Am	1	25,29
Q147H	UV	1 Af Am	0	29
A248T	UV	1 Af	1	29,40
N289H	P*	1 Af	11	29,40
Q713L	UV	1 Af Am	0	29
L929S	UV	1 Af Am, 2 Af	6	29,40
S976I	UV	1 Af Am	3	29
N987I	UV	1 Af Am, 2 Af	6	29,40
N991D	P*	1 Af	9	29,40
S1172L	UV	2 Af Am	4	29
C1290Y	P*	1 Af Am	2	29
Q1396R	UV	1 Af Am	8	29
T1414M	P*	1 Af	4	29,40
D1420Y	P	1 Af Am	113	29
D1781G	UV	1 Af Am	0	29
N1880K	P*	4 Af Am	4	29
N1880R	UV	1 Af	0	40
T1980I	UV	1 Af Am	0	29
H2074N	P*	2 Af Am	2	29
H2116R	UV	2 Af Am	7	29
K2339N	UV	4 Af Am	8	25,29
Q2384K	UV	1 Af, 1 Af Am	7	25,29,40
H2440R	UV	6 Af Am	9	25,29
A2466V	P*	8 Af Am	24	29

TABLE 2 (continued)

BRCA2 mutations	Effect	No. Af Ances Fam	No. non-Af Ances Fam	References
S2835P	UV	1 Af Am	2	29
I2944F	P*	9 Af Am	6	29
S3020C	UV	1 Af Am	0	29
M3118T	UV	1 Af Am	0	29
G3212R	UV	1 Af Am, 2 Af	0	29,40
V3244I	UV	3 Af Am	8	29
T3357I	UV	1 Af Am	0	29
I3412V	P	15 Af Am	74	29
IVS6-19 C>T	UV	2 Af Am, 2Af	9	29,40
IVS11+73 T>A	P*	1 Af	0	40
IVS18+109 G>A	UV	2 Af	0	40
IVS20-36 C>G	UV	1 Af	0	40
IVS24-T	UV	1 Af	0	40
IVS26+10T>G	UV	1 Af Am	0	29
IVS26+106 delT	P*	1 Af Am	0	29,40

Abbreviations as in Table 1; P*, polymorphism in 21 African controls [29,36]

Table 3

Frequency of BRCA Mutation among African American Families with at least two cases of female breast cancer or female breast and ovarian cancers in first degree relatives[a]

	BRCA1			BRCA2		
	Total no. families	**No. families**	**%**	**Total no. families**	No. families	**%**
Breast cancer without ovarian cancer (any age)	39	4	10	54	3	6
Breast cancer with ovarian cancer (any age)	8	3	38	12	4	33
Breast cancer < age 50[b] and at least one first degree relative with breast cancer (no ovarian ca)	25	4	16	35	3	9
Breast cancer <age 50[b] or ovarian cancer (any age) and at least one first degree relative with breast or ovarian cancers (any age)	7	3	43	10	2	20

[a]Data from references 25 and 32. [b]Proband age or median family age

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BRCA2 MUTATIONS IN AFRICAN AMERICANS

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Specific mutations in the breast-ovarian cancer susceptibility gene *BRCA2* have been associated with different ethnic groups. The entire coding regions and flanking introns of *BRCA2* are being examined for germline mutations in African American breast cancer patients from 75 families at high-risk of hereditary breast cancer. To date about 75% of the gene has been screen for mutations by single stranded conformational polymorphism and the protein truncation test, and confirmed by DNA sequencing of variants. Six pathogenic, protein-truncating *BRCA2* mutations have been identified. Two frameshift deletion mutations, 4088delA or 8643delAT, were detected in female breast cancer patients diagnosed at an early age (<40 years) with no reported family history. Frameshift mutation 2001del4 was observed in a woman with early onset breast cancer from a family with one other case of breast cancer. A patient with ovarian cancer, from a family with multiple cases of breast and ovarian cancers, carried the frameshift mutation 4075delGT. Frameshift insertion mutation 2816insA was detected in a male breast cancer patient. Frameshift mutation, 1991del4, was observed in a male breast cancer patient from a family with one other case of breast cancer. A *BRCA2* missense variant, His2395Leu, of unknown functional significance was identified. Numerous polymorphisms and noncoding variants, which are probably not disease related, were also observed in the *BRCA2* gene. Determining the spectrum of *BRCA2* mutations in African Americans is important for genetic testing and genetic counseling of African Americans. A large number of different pathogenic *BRCA2* mutations are observed in the African American population; many of these protein-truncating mutations have not been reported in other populations. The numerous, distinct pathogenic mutations in *BRCA2* observed in African Americans reflect the high level of genetic variation in people of African ancestry. Because of this broad spectrum of distinct mutations, genetic testing for *BRCA2* mutations needs to involve the entire coding and flanking sequences in high-risk patients.

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Imbalance in wild-type hMSH2:hMLH1 protein ratio in lymphocytes identifies colorectal cancer (CRC) patients with hereditary nonpolyposis colon cancer (HNPCC) traits. *Z.P. Gao[1], Z.Q. Gao[1], S. Burkholder[1], T. Zhang[1], J.Z. Fields[2], A. Kairo[1], S. Ehrlich[1], B.M. Boman[1].* 1) Medical Genetics, thomas jefferson university, philadelphia, PA; 2) CA*TX Inc., Gladwyne, Philadelphia, PA 19035

Identifying carriers of HNPCC traits can save lives and health care dollars. However, current genetics tests for HNPCC is costly and time consuming. Therefore, the need is to develop a more feasible assay to detect a germline HNPCC-causing mutation, particularly one that is sensitive, rapid, easy & inexpensive. Our approach to the problem of detecting HNPCC was to develop a quantitative immunoassay for wild-type DNA mismatch repair (MMR) protein levels (hMSH2 and hMLH1). Mutations in hMSH2 and hMLH1 account for the vast majority (>90%) of detectable germline mutations in HNPCC kindreds. And most (>70%) germline hMSH2 and hMLH1 mutations lead to a truncated protein product. We hypothesized that cells carrying a germline, truncation-causing, hMSH2 or hMLH1 mutation will have a 50% reduction in the corresponding full-length protein product. To test proof of principle for our assay, we used western blot analysis to estimate hMSH2 and hMLH1 protein levels in lymphoblastoid cell (WBC) lines from CRC patients. We tested 42 WBC lines established from CRC patients in our Familial Colorectal Cancer Registry and 8 WBC lines from healthy, unaffected individuals. Western blots were done using antibodies against the carboxyl end of both hMSH2 and hMLH1 proteins. All of the samples from healthy unaffected individuals had, on western blots, a) clearly identifiable bands for hMSH2 and for hMLH1 and b) nearly identical hMSH2/hMLH1 ratios. In 7 of the 42 WBC lines from CRC patients, we found decreased expression of hMSH2 or hMLH1 on western blot analysis and all 7 of these, subsequently, showed evidence of a mutation in corresponding MMR gene. Moreover, where DNA was available from fresh lymphocytes(5 of the 7), we found evidence for the mutation. Together these data demonstrate i) that our assay can be used to identify individuals with the HNPCC trait and ii) that this can be done practically and inexpensively.

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Identification and characterisation of the familial cylindromatosis gene. *G.R. Bignell[1], W. Warren[2], S. Seal[2], M. Takahashi[2], E. Rapley[2], R. Barfoot[2], P. Biggs[2], A. Ashworth[2], M. Stratton[2].* 1) Team 78, The Sanger Centre, Hinxton, Cambridgeshire, UK; 2) Cancer Genetics Section, Institute of Cancer Research, Sutton, Surrey, UK.

We report the cloning of the gene for Familial Cylindromatosis (*CYLD*). Familial Cylindromatosis is an autosomal dominant genetic predisposition to multiple tumours of the skin appendages. The susceptibility gene has previously been localised to chromosome 16q12-q13 and has the genetic attributes of a tumour suppressor gene/recessive oncogene. The critical evidence for involvement of the *CYLD* gene in familial cylindromatosis is the identification of 21 germline mutations. We have also identified six somatic mutations, five from familial cylindromas and one from a sporadic cylindroma. All of the germline and somatic mutation are predicted to cause early protein termination. The *CYLD* gene has also been screened through a bank of other tumours, however, no mutations were detected from this set. Analysis of the protein sequence reveals three regions with homology to Cytoskeletal Associated Protein-Glycine conserved (CAP-GLY) domains, found in proteins that co-ordinate the attachment of organelles to microtubules, and homology to the catalytic domain of Ubiquitin Carboxy-terminal Hydrolase type 2.

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Functional evidence for multiple tumor suppressor genes on the short arm of chromosome 8. *G. Chenevix-Trench[1], J. Arnold[1], P. Wilson[1], D. Trott[2], A. Cuthbert[2], R. Neubold[2].* 1) Queensland Inst Medical Res, Brisbane, Australia; 2) Brunel University, Uxbridge, UK.

The short arm of chromosome 8 frequently undergoes loss of heterozygosity in many different solid tumors, and homozygous deletions have been reported in prostate and squamous cell carcinomas. We have previously provided functional evidence of a tumor suppressor gene on this chromosome by the transfer of chromosome 8 into colorectal cancer cell lines by microcell fusion. Spontaneous deletions of the donor chromosome allowed us to map the location of the putative tumor suppressor region to a 5.2 Mb region at 8p22-23. We have now transfered chromosome 8 into ovarian (HEY) and breast (21MT and T47-D) cancer cell lines. HEY/8 hybrids containing all of the donor chromosome 8 grew normally in vitro but showed significantly reduced tumor formation in athymic mice compared to those that only contained the long arm of the donor chromosome. Analysis of hybrids containing only part of the donated 8p have allowed us to map two regions of suppression at 8p12-21 and 8p23. We have also obtained 17 21MT/8 and 5 T47-D/8 hybrids. All of these hybrids share in common the exclusion of several regions on the short arm of the donor chromosome. This suggests that they might contain tumor suppressor genes that convey a strong selective disadvantage in vitro. These data suggest that there are several tumor suppressor genes on the short arm of chromosome 8 and efforts are underway to narrow down their location prior to gene identification.

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Towards identification of a senescence-associated gene / tumor suppressor gene (TSG) in the NRC-2 locus in human chromosome band 3p14. *B. Opalka[1], W. Bardenheuer[1], G. Marquitan[1], N. Werner[1], K. Juelicher[1], H. Topal[1], I. Horikawa[2], J.C. Barrett[2], J. Schuette[1].* 1) Innere Klinik (Tumorforschung), Universitaet (GH) Essen, Essen, NW, Germany; 2) NIEHS, Research Triangle Park.

Chromosomal alterations in human chromosome region 3p14 have been found in numerous tumor entities including lung cancer (LC) and renal cell carcinoma (RCC). Results of functional investigations as well as data concerning structural aberrations suggest the presence of at least one TSG in 3p14. The FHIT gene in 3p14.2 and the WNT5A gene in 3p14.3-21 have been shown to reveal TSG function in vitro and/or in animal models while mutations of these genes have been rarely detected in LC or RCC. We have previously established functional complementation assays using a YAC contig covering chromosomal band 3p14 and neighboring regions. Following retrofitting of YACs for the introduction of a mammalian selectable marker we established the YAC transfer by spheroplast fusion into a human RCC line showing a cytogenetically detectable deletion within 3p13-23. Using this approach we identified a 530 kb YAC clone within 3p14.2 which induced cellular senescence in vitro and reveals sustained suppression of tumorigenicity of transduced RCC cells in nude mice. This activity which maps differently from the FHIT gene and the WNT5A gene defines a novel TSG locus, NRC-2, in 3p14. Genomic sequencing was performed using PAC clones. Including data from the human genome project 60-80% of the entire YAC sequence are available now. Primers were synthesized corresponding to 6 ESTs and 15 predicted exon sequences as well as for 8 exon sequences identified in exon trapper experiments and are currently used to screen different cDNA libraries. These efforts should allow the isolation of the gene(s) responsible for the NRC-2 locus activity. Supported by Deutsche Forschungsgemeinschaft and foundation VerUm.

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Two deletions in regions 6q22-23 and 6q25-27 are associated with immortalization of SV40 transformed cells. *J. Liu, A.K. Sandhu, N. Rane, R.S. Athwal.* Department Of Pathology,Fels institute, Temple University School of Med. Philedelphia, PA.

We have applied a 'Functional-Potional' approach to identify cell senescence genes on human chromosomes. Microcell mediated transfer of a gpt tagged intact human chromosome 6 or a part of the long arm(6q14-qter) restored senescence in human (Sandhu et al. 1994, PNAS 91: 5498-5502) and mouse SV40 immortalized fibroblasts. Segregation of the transferred chromosome 6, with the loss of gpt tag, led to the resumption of indefinite cell proliferation. While microcell hybrids were maintained in the MX selection medium, for the retention of the donor chromosome, immortal revertant clones arose among senescent cells. Reversion to immortal growth could result from the loss of the expression of the senescence gene due to a mutation or a deletion. Analysis of the revertant clones for the loss of the DNA markers, mapped in the region 6q22-qter, identified two deletions in the region 6q22-27. The results of this study revealed two independent deletions located at 6q22-23 and 6q25-27, suggesting the location of two senescence genes on the long arm of chromosome 6. In order to further define the position of the senescence gene(s), we have identified YAC and BAC clones corresponding to the deleted markers, by human genome data base search. A high resolution physical map of the region containing the senescence gene has been developed. Candidate BAC clones will be tested for the restoration of senescence by introduction into SV40 immortalized human and rodent cells.

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BRCA2 mutations in African Americans. *Y.M. Kanaan[1], E. Kpenu[1], K. Utley[1], L.C. Brody[2], G.M. Dunston[1], C. Whitfield-Broome[1].* 1) Howard University College of Medicine, Departments Microbiol., Biochem.& Mol. Biol.; Cancer Center, Humam Genome Center, Washington, DC.20059; 2) National Human Genome Res. Inst., Bethesda MD 20892-4442.

Since the identification of the BRCA2 breast-ovarian cancer susceptibility gene, mutation analyses have been carried out in various populations revealing ethnic-specific mutations. In order to identify BRCA2 mutations in African Americans, seventy-five breast cancer patients from families at high risk of hereditary breast cancer were studied. The entire coding regions and flanking introns of BRCA2 have been screened for germline mutations by single stranded conformational polymorphism or the protein truncation test, followed by DNA sequencing. Eight protein truncating, pathogenic mutations have been detected. Four (1991delATAA, 1993delAA, 2001delTTAT, 8643delAT) of the eight pathogenic mutations observed in African Americans have not been previously described. Therefore, half of the pathogenic mutations observed are unique to African Americans. The other four pathogenic mutations (1882delT, 2816insA, 4075delGT, 4088delA) detected in African Americans have been previously reported in Caucasians. Two of the pathogenic mutations, 1991delATAA and 2816insA, were detected in male breast cancer patients, consistent with previous studies showing an association of BRCA2 with male breast cancer. One-half of the pathogenic mutations were identified in women diagnosed with breast cancer under the age of 40 with or without a family history of the disease. One rare missense variant of unknown functional significance was detected. Numerous polymorphisms and non-coding variants were observed. Considering our work and that of others, many different pathogenic mutations and many variants of unknown significance are observed in African Americans; therefore, BRCA2 genetic testing in high risk African American families needs to involve the entire coding and flanking sequences of the gene. This work was supported by the U.S. Army Medical Research and Materiel Command under DAMD17-98-1-8106 and in part by the Komen Found.

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ORIGINAL INVESTIGATION

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BRCA1 mutations in African Americans

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Abstract The breast cancer predisposing gene, *BRCA1*, was analyzed for germline mutations in 45 African American families at high-risk for hereditary breast cancer. Patients were considered high-risk if they had a family history of the disease, early onset breast cancer, bilateral breast cancer, or breast and ovarian cancer. The entire *BRCA1* coding and flanking intron regions have been examined by single stranded conformation polymorphism analysis followed by sequencing of variant bands. Eleven different *BRCA1* germline mutations/variations were identified in 7 patients from the 45 high-risk families. Two pathogenic, protein-truncating mutations were detected in exon 11. A ten base pair tandem duplication, 943ins10, was present in a woman with breast and ovarian cancer whose first-degree relatives had prostate cancer. A four base pair deletion, 3450del4, was detected in a breast cancer patient with five cases of breast cancer in the family; two of the proband's sisters with breast cancer also carried the same mutation. Four amino acid substitutions (Lys1183Arg, Leu1564Pro, Gln1785His, and Glu1794Asp) and four nucleotide substitutions in intron 22 (IVS22+78 C/A, IVS22+67 T/C, IVS22+8 T/A and IVS22+7 T/C) were observed in patients and not in control subjects. One early onset breast cancer patient carried five distinct *BRCA1* variations, two amino acid substitutions and three substitutions in intron 22. An amino acid substitution in exon 11, Ser1140Gly, was identified in 3 different unrelated patients and in 6 of 92 control samples. The latter probably represents a benign polymorphism.

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Introduction

Mutations in single genes may account for 3–8% of all breast cancers (Easton 1994; Eeles et al. 1994). Early onset disease is common in hereditary breast cancer families. The incidence of breast cancer among blacks is higher than whites below the age of fifty (Ries et al. 1998). Based on the linkage studies of King and colleagues (Hall et al. 1990), the first breast cancer predisposing gene *BRCA1* was identified by Miki et al. (1994). From 16–52% of all cases of a family history of breast cancer and 40–81% of cases with a family history of breast and ovarian cancer have been linked to germline mutations in *BRCA1* (Couch et al. 1997; Ford et al. 1998). Since its isolation less than five years ago, more than 600 distinct germline mutations have been described in *BRCA1* (Breast cancer information core, BIC 1998). Specific breast cancer predisposing mutations in *BRCA1* have been associated with different ethnic groups. For example, the *BRCA1* 185delAG allele is present in nearly 1% of Ashkenazi Jews (Struewing et al. 1995). Among Dutch breast cancer patients, 36% of *BRCA1* mutations are large deletions (Petrij-Bosch et al. 1997). Most studies of hereditary breast cancer have included few African Americans (Castilla et al. 1994; Futreal et al. 1994; Miki et al. 1994). In order to determine the spectrum of germline *BRCA1* mutations in African Americans, the entire coding region and all flanking introns were examined in 45 breast cancer patients from families at high risk of hereditary breast cancer. Thus, this study represents one of the largest collections of high-risk African Americans whose *BRCA1* coding sequences have been completely scanned for mutations.

Materials and methods

Subjects

As part of an ongoing, population-based epidemiological study of breast cancer in African Americans, 45 high-risk families were selected from among 250 patients seen at the Howard University Cancer Center (Table 1). High-risk criteria were multiple cases (including first-degree, second-degree, and distant relatives in the same lineage) of breast cancer or multiple cases of breast and ovarian cancer per family; breast cancer with early age of onset (≤ 40 years); bilateral breast cancer; breast and ovarian cancer in the same individual; or male breast cancer. Of the multiple case families, none had both breast and ovarian cancer; there were nine families with four or more cases of breast cancer (mean age of diagnosis of individual = 47 years). There was no upper or lower age cutoff for breast cancer cases. Breast cancer was verified by the original pathology report for all probands, but not for all affected relatives. Blood samples from the 45 high-risk breast cancer patients (probands) as well as 92 ethnically matched, population-based community controls were utilized for genomic analysis. The study was approved by the Howard University Institutional Review Board.

Table 1 African American breast cancer families at high risk for breast cancer predisposing mutations

Case category for selection	Mean age at diagnosis of individual (years)	Number of cases (% total cases)
Multiple-case families (≥ 3[a])	50	18 (40%)
Multiple-case families (2[a])	44	14 (31%)
Early onset (≤ 40 years) breast cancer	35	8 (18%)
Bilateral breast cancer	48	2 (4%)
Male breast cancer	52	2 (4%)
Breast and ovarian cancers[b]	50	1 (2%)
Total cases	46	45 (100%)
Cases tested ≤ 40 years	36	19 (42%)

[a] Number of breast cancer cases per family, including first-degree, second-degree, and distant relatives
[b] Same individual with both breast and ovarian cancers

Single stranded conformation polymorphism (SSCP)

DNA was extracted from cryopreserved peripheral blood lymphocytes using a standard phenol/chloroform method (Sambrook et al. 1989). Polymerase chain reaction amplification was carried out in a total reaction volume of 15 µl containing 40 ng of genomic DNA as template, 0.67 µM each of exon-specific forward and reverse primers, 0.67 mM each of dATP, dCTP, dGTP and dTTP; 1.5–3.3 mM $MgCl_2$, 33 mM Tris-HCl (pH 8.4), 83 mM KCl, 10 µCi α[^{32}P]dCTP, and 1.25 U of *Taq* polymerase (Life Technologies). Primers are described in the BIC. Initial denaturation was at 97 °C for 5 min followed by 30 cycles of denaturation at 95 °C for 20 sec, annealing at 50 °C for 20 sec and extension at 72 °C for 2 min. Annealing temperatures were adjusted according to the melting temperature of each primer pair.

SSCP analysis was an adaptation of the method previously reported by Orita et al. (1989). ^{32}P-labeled PCR products were resolved by electrophoresis on non-denaturing 0.5 × MDE (AT Biochem) gels in 0.60 × TBE buffer for 14–18 h at room temperature and constant wattage of 8 W. The gels were dried at 80 °C for 1 h and exposed to film for 1–12 h.

DNA sequencing

Variant bands detected on the SSCP gels were excised from the gels, re-amplified, purified and sequenced. Fluorescent dye terminator cycle sequencing was performed using the same forward and reverse primers as in the PCR reaction; products were analyzed with an automated sequencer (models 310, 373A and 377, Applied Biosystems) Three independent PCR reactions of each variant were sequenced to eliminate false positives due to *Taq* polymerase errors.

Table 2 *BRCA1* sequence alterations in African American breast cancer patients. (*BR* breast, *OV* ovarian, number in *parentheses* indicates number of breast and ovarian cancer cases per family)

Case number	Cancer type	Age at diagnosis (years)	Exon	Nucleotide[a]/ codon	Mutation	Amino acid change	Designation
BC003	BR +	50	11	943/275	10 base insertion: AGCCATGTGG	Frameshift stop at codon 289[b]	943ins10
	OV (1)	46					
BC022	BR (5)	48	11	3450/1111	4 base deletion: CAAG	Frameshift stop at codon 1115[b]	3450del4
BC025	BR (3)	56	11	3667/1183	A to G	Lys to Arg	K1183R
			11	3537/1140	A to G	Ser to Gly	S1140G[c]
BC001	Early onset BR (1)	33	16	4810/1564	T to C	Leu to Pro	L1564P
			22	5474/1785	G to T	Gln to His	Q1785H
			Intron 22	–	C to A	Non-coding	IVS22+78 C/A
			Intron 22	–	T to C	Non-coding	IVS22+67 T/C
			Intron 22	–	T to A	Non-coding	IVS22+8 T/A
BC040	BR (2)	42	22	5501/1794	G to T	Glu to Asp	E1794D
					T to C	Non-coding	IVS22+7 T/C
					C to A	Non-coding	IVS22+78 C/A
BC023	BR (3)	63	11	3537/1140	A to G	Ser to Gly	S1140G*c*
BC044	BR (2)	42	11	3537/1140	A to G	Ser to Gly	S1140G*c*

[a] Numbering starting with the first nucleotide in the 5′-untranslated region of *BRCA1* cDNA (GenBank accession no. U14680)
[b] Including newly inserted/deleted amino acids and stop codon
[c] This alteration was detected in 6 out of 92 control subjects not selected for disease

Family BC003

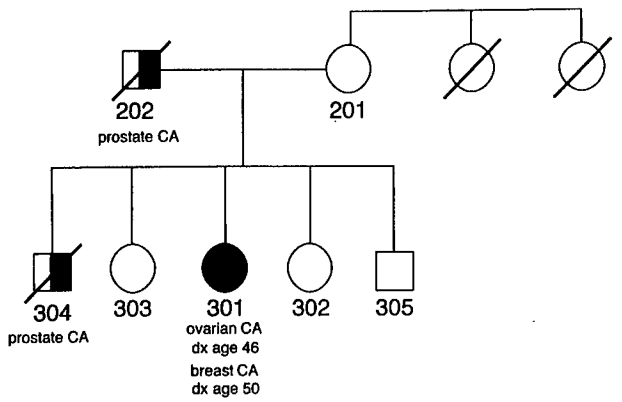

Fig. 1 Pedigree of family BC003, which carries the BRCA1 943ins10 mutation. At the time of the study, the mother (201) was over 85 years of age and the siblings of 301 were over 40 years of age. *Filled circle*, proband diagnosed with breast and ovarian cancer. *Half-filled squares*, males diagnosed with prostate cancer

Family BC022

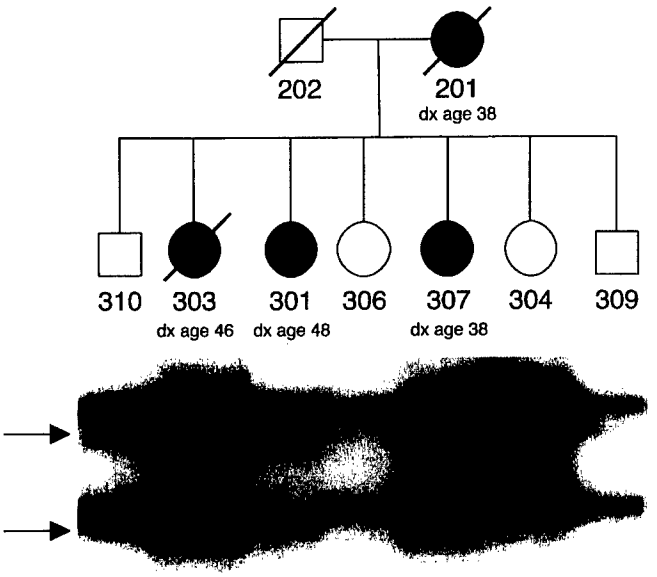

Fig. 2 Pedigree and mutation analysis of family BC022, which carries the 3450del4 mutation. Not shown is a maternal fourth degree cousin diagnosed with breast cancer at the age of 33 years. She was not available for study. Some of the pedigree structure has been altered to protect the identity of the family. *Lower panel*, autoradiogram of SSCP analysis of exon 11. Brothers 310 and 309, and sisters 306 and 304 exhibit 2 normal SSCP bands. Sisters 303 and 307, who were diagnosed with breast cancer (*filled circles*), show two normal bands and two variant bands (*arrows*). Proband 301 reveals one normal and two variant bands

Results and discussion

Two patients were found to carry pathogenic, protein-truncating mutations. A ten base pair tandem duplication (AGCCATGTGG), designated 943ins10, was detected in a patient with breast and ovarian cancer, BC003 (Table 2). No breast cancer was reported in the mother (age 87 years), or sisters (ages 44, 53 years) of the proband; however, the father and one of two brothers of this patient have prostate cancer (Fig. 1). This frameshift mutation is predicted to produce a protein termination at amino acid residue 289, thus presumably deleting 85% of the protein. The same ten base pair insertion was reported in a family from the Ivory Coast (Stoppa-Lyonnet et al. 1997) and in three families of African ancestry (Arena et al. 1996, Arena et al. 1997; Mefford et al. 1999).

In the multiple case family, BC022, a four base pair deletion of CAAG was detected, which produced a frameshift mutation (Table 2). This frameshift mutation is predicted to result in a protein truncation at codon 1115, putatively deleting 40% of the protein. In addition to the proband, the mutation was detected in two sisters affected with breast cancer, but not in two sisters or two brothers unaffected with breast cancer (Fig. 2). The BC022 proband, 301, exhibited three bands on SSCP analysis (Fig. 2), instead of the expected four for a heterozygous mutant (2 normal and 2 mutant bands, as in 303 and 307). Sequencing of the amplified DNA without separation on the MDE gel revealed heterozygosity for the 3450del4 mutation in the proband. The 3450del4 mutation has been reported to the BIC in one Norwegian and two Canadian patients whose ethnicity is unknown. Neither frameshift mutation was found in the 92 control subjects.

Four amino acid substitutions (Lys1183Arg, Leu1564Pro, Gln1785His, Glu1794Asp) were identified in breast cancer patients but not in 92 control subjects (Table 2). One patient (BC001) with early onset breast cancer exhibited two different amino acid substitutions (Leu1564Pro, Gln1785His) and three additional intron 22 substitutions. Glu1794 is conserved in mouse, dog and rat *BRCA1* (Abel et al. 1995; Bennett et al. 1995; Bennett et al. 1999; Chen et al. 1996; Sharan et al. 1995; Szabo et al. 1996) Therefore, the substitution of Asp for Glu at codon 1794 may affect the function of the protein. In contrast, for each of the other amino acid substitutions described (Lys1183Arg, Leu1564Pro, Gln1785His, Ser1140Gly), the human wild type amino acid is not conserved in one of the three species. Further functional tests are needed to determine the biological significance of these amino acid substitutions, none of which are in the conserved region of the BRCA1 C-terminal (BRCT) domain (Callebaut and Mornon 1997). The Lys1183Arg substitution has been reported in another African American breast cancer patient (Shen et al. 1998). Three out of 45 patients and 6 out of 92 African American control subjects exhibited a change of Ser1140Gly (Table 2). This amino acid substitution has been observed in other African American breast cancer patients (Gao et al. 1997; Shen et al. 1998) and is probably a benign polymorphism. Four nucleotide substitutions in a non-coding region (intron 22; IVS22+78 C/A, IVS22+67 T/C, IVS22+8 T/A, and IVS22+7 T/C) were detected in two patients but not in the controls (Table 2). Two of these intronic variations (IVS22+67 T/C, IVS22+8

T/A) were detected in African American patients and controls in another study (Arena et al. 1998).

In the present study, the following alterations were observed: two pathogenic, protein-truncating mutations, one in a patient with breast and ovarian cancer and one in a patient from a family with five cases of breast cancer; four amino acid substitutions; one amino acid polymorphism; and four substitutions in non-coding regions (introns). Our data, coupled with that of other investigators (Castilla et al. 1994; Futreal et al. 1994; Miki et al. 1994; Arena et al. 1996; Arena et al. 1997; Arena et al. 1998; Gao et al. 1997; Stoppa-Lyonnet et al. 1997; Ganguly et al. 1998; Shen et al. 1998), reveal a large number of distinct pathogenic mutations and variations among African Americans. Most of these variations have not been reported among Caucasians (BIC 1998). The 943ins10 mutation has been observed in five different families who appear to have a common, distant African ancestor (Mefford et al. 1999). This diverse spectrum of variations/mutations is consistent with the high level of genetic diversity in people of African ancestry (Jorde et al. 1998).

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Am. J. Hum. Genet. 65:575–578, 1999

Evidence for a BRCA1 Founder Mutation in Families of West African Ancestry

To the Editor:

Inherited mutations in the BRCA1 gene (MIM 113705; GenBank U14680) (Miki et al. 1994) are less common among breast cancer patients of African American ancestry than among those of white ancestry. For example, in a population-based series of breast cancer patients from North Carolina, the prevalence of BRCA1 mutations was 3.3% among white women and 0% among African American women (Newman et al. 1998). Nonetheless, inherited BRCA1 mutations have been identified in families of African and African American ancestry at high risk of breast cancer (Gao et al. 1997; Stoppa-Lyonnet et al. 1997; Panguluri et al. 1999 [in press]). To provide effective genetic testing for African American families at high risk for breast and ovarian cancer, it would be helpful to identify ancient BRCA1 mutations of African origin analogous to ancient mutations in other populations (Simard et al. 1994; Peelen et al. 1997; Petrij-Bosch et al. 1997). Here we have described one apparently ancient, African BRCA1 mutation.

BRCA1 mutation 943ins10 was detected in breast cancer patients from the Ivory Coast (Stoppa-Lyonnet et al. 1997), the Bahamas, and the United States (Arena et al. 1997; Panguluri et al. 1999 [in press]) (fig. 1). To confirm the identity of the mutation for the five probands and their relatives, the critical region of BRCA1 was genotyped by fluorescent sequencing with dRhodamine-dye terminators (Applied Biosystems). Primers 5′-GGAATTAAATGAAAGAGTATG-AGC-3′ and 5′-CTTCCAGCCCATCTGTTATGTTG-3′ revealed the heterozygous frameshift mutation 943ins10, a 10-bp insertion in exon 11, leading to a stop at codon 289. The mutation is a tandem duplication, in a repeated-sequence motif, that could have occurred at any site between BRCA1 nucleotides 926 and 943 (fig. 2). The notation "943ins10" designates the most-3′ site of insertion possible (Antonarakis et al. 1998). The 943ins10 variant can be easily detected on agarose gel by amplification of genomic DNA or cDNA with BRCA1 primers 5′-CTGCTTGTGAAT-TTTCTGAGACGG-3′ and 5′-TGCTGTAATGAGC-TGGCATGAG-3′ under standard conditions. Wild-type BRCA1 sequence yields a product of 184 bp, and 943ins10 yields a product of 194 bp.

Genotypes of relatives in these five families were consistent with BRCA1 943ins10 being a founder mutation of African origin. Nine markers within and flanking BRCA1 were genotyped (Genome Database): D17S1325, D17S1326, and D17S1327 (5′ of BRCA1); D17S1323 (intron 12), D17S1322 (intron 19), and D17S855 (intron 20); and D17S1321, D17S1320, and D17S1185 (3′ of BRCA1) (Neuhausen et al. 1996; Smith et al. 1996). The 943ins10 mutation occurred on a single haplotype spanning D17S1320–D17S1326 (fig. 1), a distance of ~700 kb.

The families inheriting BRCA1 943ins10 were from widespread locales of Africa and the African diaspora: the Ivory Coast, the Bahamas, the southeastern United States, and Washington, DC. The families are not recently related, and the four families in North America can trace their history in this hemisphere to the slavery period. The length of the 943ins10 nonrecombinant BRCA1 region is similar to the length of the shared region flanking the BRCA1 mutation 185delAG. Hence, the ages of these mutations may be comparable (Bar-Sade et al. 1998). The shared BRCA1 region flanking 943ins10 is shorter than the BRCA1 regions flanking 5382insC or 2800delAA, so the African mutation is probably older than these European mutations (Neuhausen et al. 1996; Friedman et al. 1995). West Africans were brought to North America as slaves between 1619 and 1808. Hence, the social history of the families studied indicates that the mutation is >200 years old and could be much more ancient.

Figure 1 indicates additional, known cases of breast and ovarian cancer in each family. In families UM94003 and UM95027, mothers of probands were affected. In families UM96034 and HU003, in which mothers were not affected, the 943ins10 allele was inherited from the father. Age at breast and ovarian cancer diagnosis was ≤50 years for all probands and affected relatives. Family IC564 includes four women with breast or ovarian cancer, all of whom live in the Ivory Coast, where breast and ovarian cancer are rare (Parkin et al. 1997). In this family, the mother of a patient with ovarian cancer remains unaffected at age 83 years, though she is likely to carry the mutation. That there are elderly carriers without cancer suggests that nongenetic factors may influence the penetrance of BRCA1 alleles in geographic regions with a low background risk for breast cancer.

The geographic distribution of 943ins10 in North America is intriguing and is not completely known. BRCA1 943ins10 occurred in 3 of 96 African American patients seen at the University of Miami, who had breast cancer diagnosed at an early age, and in 1 of 55 African American patients seen at Howard University in Washington, DC, who had breast cancer diagnosed at an early age or who had families with a high incidence of breast cancer. However, in the population-based Carolina Breast Cancer Study, the mutation did not appear among 263 African American breast cancer patients, 50% of whom were aged <50 years and 50% of whom were aged ≥50 years at diagnosis (Newman et al. 1998). The 943ins10 allele has not been observed in any patients

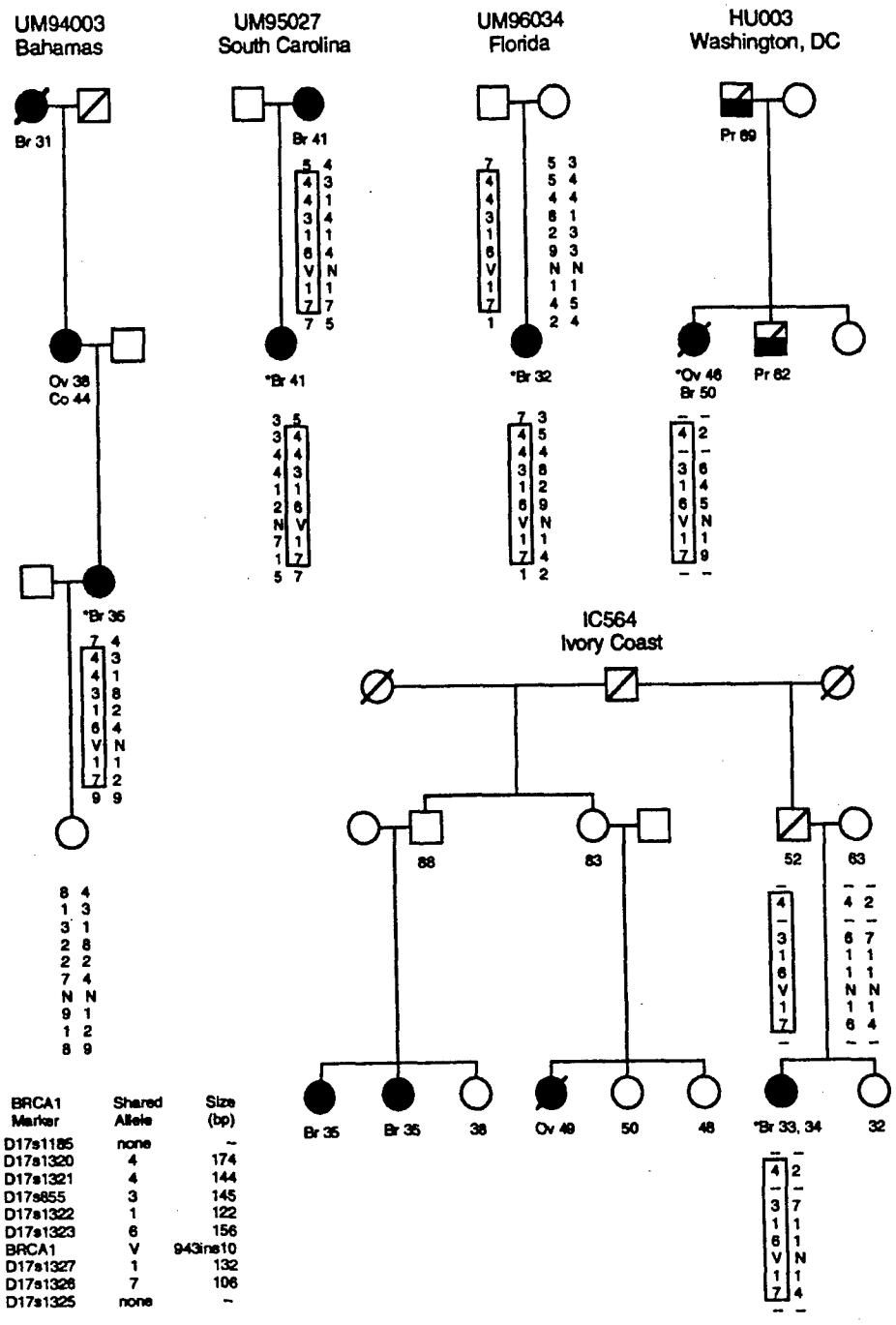

Figure 1 Pedigrees of families carrying the BRCA1 943ins10 mutation. Affected individuals are indicated by a blackened symbol, and probands are denoted by an asterisk (*). The shared haplotype segregating with 943ins10 is boxed. Haplotypes of the fathers of probands in families UM96034 and IC564 have been reconstructed.

with breast cancer who identify their ancestry as solely European.

The migration patterns of African Americans and, hence, the current areas of residence of African American families, may explain the difference, among clinical centers, in the prevalence of the mutation. To determine, among African American women, the proportion of inherited breast or ovarian cancer attributable to BRCA1 943ins10, we would like to encourage testing for this mutation among African American breast and

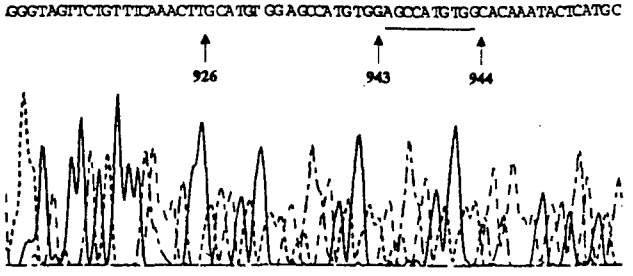

Figure 2 Sequence of the BRCA1 943ins10 mutation. A duplication and insertion of 10 bp causes a frameshift and premature truncation at amino acid 289.

ovarian cancer patients from various regions of the United States. Given the increasing incidence of and higher mortality from breast cancer among African American women, it would be useful to obtain as much information as possible about the roles of BRCA1 and BRCA2 in this population.

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Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

GenBank, http://www.ncbi.nlm.nih.gov/Entrez (for BRCA1 [U14680])

Genome Database, http://gdbwww.gdb.org (for D17S1325, D17S1326, D17S1327, D17S1323, D17S1322, D17S855, D17S1321, D17S1320, and D17S1185)

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#1783 Distinct mutation patterns and clonal instability of breast-cancer-associated alleles of the *HRAS1* minisatellite locus. Ding, S., Larson, G.P., Foldenauer, K., Zhang, G., and Krontiris, T.G. *Division of Molecular Medicine, Beckman Research Institute of the City of Hope National Medical Center, Duarte, CA 91010.*

The *HRAS1* minisatellite is composed of tandem copies of a polymorphic 28-bp repeat: dozens of rare alleles are derived from 4 common progenitors. We undertook a detailed analysis of the repeat interspersion patterns comprising the minisatellite in rare (high-risk) and common (low-risk) alleles of breast cancer patients and cancer-free controls to obtain clues about both the mechanism of pathogenicity and the process of mutation. DNA sequence of 130 alleles showed that breast-cancer-associated mutations arose as a consequence of both replication errors and gene conversions. Unlike mutations at other VNTRs, high-risk mutations of the *HRAS1* minisatellite did not demonstrate positional polarity. Instead, most mutations occurred at three hotspots, with replication errors confined to one hotspot, gene conversions to a second, and a mixed pattern of mutation at the third. Mutations even one repeat unit larger or one unit smaller than their progenitors show precipitous drops in stability when cloned in bacteria. Thus, the cancer association of this minisatellite locus may have two features. First, the variety of mutations suggests that the minisatellite may serve as a reporter for a broad-based group of mutational mechanisms and candidate mutator loci. Second, mutations may alter the chromosomal stability of the locus. The implications of this finding for tumorigenesis, as well as ongoing studies on the effects of replication, repair and recombination pathways in lower organisms on minisatellite stability, will be discussed.

#1784 Alcohol dehydrogenase 1 is expressed in human breast epithelial cells and is down-regulated in breast cancer. Atkins, T.A., Weisz, J.P., Beneski, J.T., Triano, E.A., and Slusher, L.B. *Penn State–Geisinger Health Systems, Hershey PA 17033 and West Chester University, West Chester, PA. 19383.*

Alcohol dehydrogenase 1 (ADH1) has three functions relevant to carcinogenesis: i) it catalyzes the first step in the biogenesis of retinoic acid, a promoter of cellular differentiation and a suppressor of cellular replication; ii) it oxidizes ethanol to its potentially cytotoxic aldehyde: and iii) it participates in the detoxification of 4 hydroxynonenal (4HNE), a cyto- and genotoxic lipid peroxidation product. By immunocytochemistry (ICC) and *in situ* hybridization, we have identified ADH1 in mammary epithelial cells of normal human breast parenchyma (n=6) and shown its expression to be greatly reduced or abolished in breast cancer (n=6). Findings by ICC were confirmed by immunoblot analysis. We propose that down-regulation of ADH1 aids in the progression of breast cancer by reducing the generation of retinoic acid, a tumor suppressor. To determine if the down-regulation of ADH1 contributes to cancer promotion, it will be necessary to determine when in the multi-step process of carcinogenesis ADH1 is down-regulated.

#1785 The mutational analysis of the BAP1 gene for breast and lung cancer. Shirahama, S., Ishikawa, H., Hikiji, K., Aoki, T., Amano, J. and Fujimori, M. *SRL Inc., Tokyo, Dept. of Surgery, Shinsyu Univ. Sch. of Med., Matsumoto, Japan.*

BAP1 (BRCA1 associated protein-1), which binds to the RING finger domain of the Breast/Ovarian cancer susceptibility gene product, BRCA1, has recently been identified. BAP1 is a nuclear-localized, ubiquitin carboxy-terminal hydrolase, suggesting that deubiquitinating enzymes may play a role in BRCA1 function. BAP1 locates on human chromosome 3p21.3; intragenic homozygous rearrangements and deletions of BAP1 have been found in lung carcinoma cell lines. We attempted to analyze the BAP1 gene mutatons in breast and lung cancer specimens. Total RNA was isolated from the tumor tissues. We amplified the BAP1 coding region 2.2kb, separated two regions of 1.0kb and 1.2kb, with RT-PCR and directly sequenced the BAP1. We confirmed that all samples expressed BAP1 mRNA and expression pattern were not variety. In the result of sequencing analysis, none created sequence differences unique to affected individuals. Our study suggests that BAP1 mutations are not common in breast and lung cancers and that allelic deletions of involving 3p21.3 play a crinical role in the tumorigenesis.

#1786 A candidate founder mutation of BRCA1 gene in African American breast cancer patients. Shen, D., Wu, Y., Subarrao, M.N., Bhat, H.K., Chillar, R., Vaugarna, J.V. *Charles H. Drew University of Medicine and Science, Los Angeles, CA 90059.*

Studies in Ashkenazi Jewish population and in some other populations suggest that founder BRCA1 mutations usually have the characteristic of ethnic or geographic distribution. The goal of this study is to identify the BRCA1 founder mutations and novel mutations in minority breast cancer patients. Screening of the mutations in BRCA1 hot spot regions including exons 2, 5, 11, 16 and 20 has been performed using SSCP and NIRCA methods in 107 minority breast cancer patients. We identified seven different kinds of mutations in exon 11 of BRCA1 gene. All these mutations are localized in the fourth portion of exon 11 (Fragment 3). Six out of 54 African American (AA) breast cancer patients have been detected carrying BRCA1 gene mutations. Among them, one AA breast cancer patient carried both a frameshift mutation, 3331insG and a missense mutation, 4009C→T. Another 3 missense mutations, 3537A→G, 3610G→C and 3667A→G, have been detected in AA breast cancer patients and one missense mutation, 3537A→G has been detected in 3 different breast cancer patients. Most AA breast cancer patients (5/7), who carried BRCA1 gene mutations, are young patients (age≤50). Only two missense mutations, 3667A→G and 3717C→T, have been found in 46 Hispanic breast cancer patients. In summary, our study demonstrated one missense mutation, 3537A→G in AA breast cancer patients, with high incidence (3/54, 7.4%), which may have the founder effect. Also different patterns of BRCA1 gene mutation distribution, which was characterized by a mutation cluster in BRCA1 exon 11 fragment D, have been found in AA breast cancer patients. The Hispanic breast cancer patients have low incidence of BRCA1 mutations.

#1787 *BRCA2* mutations in African American (AA) and African women with breast and/or ovarian cancer. Gao, Q[1]., Das, S.,[2] Sveen, L.,[1] Rogers, L.,[2] Isaacs, C.,[3] Tomlinson, G.,[4] Olopade, OI.[1]. *[1]Section of Hematology/Oncology, Department of Medicine; [2] Department of Human Genetics, the University of Chicago, Chicago, IL 60637; [3] Georgetown University Medical Center, Washington D.C; [4]UT Southwestern Medical Center, Dallas, TX.*

The vast majority of African Americans in the US originated from West Africa, where breast cancer is considered to be a rare aggressive disease predominantly affecting young women. Although the search for mutations in breast cancer susceptibility genes *BRCA1 /2* has been extensively conducted in various populations, the nature of *BRCA1 /2* mutations in Africa is just beginning to be revealed by the evaluation of families of combined African and European or American ancestry. To date, nine unique *BRCA1* and only one *BRCA2* mutations have been described in extended AA families with early-onset breast and/or ovarian cancer. To further explore the role of *BRCA2* in AA breast cancer patients, as well as its correlation with that in Africans, we screened 36 AA patients, including 27 selected for family history, and 40 African samples from Nigeria for *BRCA2* mutations. Four frameshift mutations, one missense mutation and six unclassified variants have been identified in AA samples. All the five mutations were novel and were found in patients with strong family history and one has been reported in three unrelated families. This result suggests that the AA population has a unique mutation spectrum in *BRCA2*, with the possibility of founder mutations. Screening for *BRCA2* mutations in African samples is currently underway.

#1788 BRCA2 mutations in African Americans. Whitfield-Broome, C., Dunston, G.M., Brody, L.C. *Howard Univ. Cancer Center & Howard U. Col. of Med. Dept. of Biochem. & Dept. of Microbiol., Washington D.C, 20059; GMMB, National Human Genome Research Institute, Bethesda, MD 20892-4442.*

Specific BRCA2 mutations have been associated with different ethnic groups. In order to determine the spectrum of BRCA2 breast cancer predisposing mutations in African Americans, fifty-five breast cancer patients from high-risk families were examined. The criteria for high risk were families with 2 or more cases of breast and/or ovarian cancer, early age of cancer onset (<40 years), breast and ovarian cancer in a single individual, bilateral breast cancer, or male breast cancer. Exons 10 and 11 are being screened using genomic DNA and the protein truncation test. To date four pathologic mutations have been observed; two in exon 10 and two in exon 11. Mutation 1991delATAA was observed in a male breast cancer patient; 2816insA was detected in another male breast cancer patient. Mutation 2001delTTAT was identified in a family with 2 cases of female breast cancer. A truncated protein was detected in a family with 4 cases of female breast cancer. Even though only one-half of the BRCA2 gene has been analyzed, we have found more pathologic BRCA2 mutations than BRCA1 mutations in this cohort of high-risk patients.

#1789 Analysis of the BRCA1 gene mutations and its implications in breast and ovary cancer in a well documentated family. Román García J, Montoriol Sabate C, Morer De Llorens I, Hilari Serra JM, García Foncillas J, de Cuevillas Matozzi F. *Clínica Ruber Internacional, Madrid, Spain (J.R.G); Clínica Universitaria de Navarra, Pamplona, Spain (J.G.F); Laboratorio Dr. Echevarne, Barcelona, Spain.*

BRCA1 is located in the chromosome 17q21. This gene is most associated with familial breast and ovary cancer syndromes. Women with the BRCA1 mutation have an 80 to 90% lifetime risk of developing breast cancer and a 40 to 50% risk of ovary cancer. A deletion or alteration in just one copy of the BRCA1 gene predisposes to breast and ovary cancer and potentially colon and prostate cancer. We have analyzed the BRCA1 gene structure in four very well studied patients. Three sisters had ovary cancer and one breast cancer. At the moment only one is alive.

Two DNA samples were obtained from paraffin-embedded tumor specimens, in the case of the dead women. In the other two cases, DNA was extracted from peripheral blood. We have sequenced the entire gene with an ABI-377 fluorescent sequencer with he dye terminators according to manufacturer's suggestions. In addition, we are studying another sister, three brothers and eight sons and daughters. In this group of people, we are searching for the founded mutation. In the four samples analyzed we have found the same mutation at the 1294 codon in the exon 11.

The mutation found originate and STOP codon and an altered protein which is related with an early ovary cancer. We are studying the rest of the family in order to asses the risk of breast and ovary cancer because there are some strong familiar clues for doing it. The mother of these four persons has a pancreas tumor,

Inheritance of Breast Cancer in African American Women: How Should We Monitor?

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Abstract

Inherited mutations in single genes account for 3-8% of all breast cancer. Most inherited breast cancer is due to the highly penetrant breast cancer predisposing genes *BRCA1* and *BRCA2*. Families at high-risk of inherited breast cancer are those with multiple cases of breast cancer in the same (maternal or paternal) lineage, one first-degree (mother, sister) relative with early-onset (<age 50) breast cancer, two first-degree relatives with breast cancer, ovarian cancer, bilateral breast cancer, or male breast cancer. Our data coupled with that of other investigators reveal a large number of distinct pathogenic mutations and variations among African Americans. Most of these variations have not been reported among Caucasians. Preliminary results reveal more *BRCA2* pathogenic, protein truncating mutations than *BRCA1* pathogenic, protein truncating mutations. This diverse spectrum of variations/mutations is consistent with the high level of genetic diversity in people of African ancestry. Due to this genetic diversity, the entire *BRCA1* and *BRCA2* coding and flanking sequences need to be tested in high-risk African American patients.

Presented at Howard University Women's Health Institute Conference: Health Issues and Concerns of Women of Color: A Call to Action. Washington DC, April, 1999.

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